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            12 "BOLOGNESI DANI P"/IN
             4 "BOLOGNESI DANI PAUL"/IN
L1          16 "BOLOGNESI DANI P"/IN OR "BOLOGNESI DANI PAUL"/IN

=> d l1,ti,1-16

L1   ANSWER 1 OF 16  USPATFULL on STN
TI     Nucleic acids encoding DP-178 and other viral fusion inhibitor peptides
       useful for treating aids

L1   ANSWER 2 OF 16  USPATFULL on STN
TI     Compounds which inhibit HIV replication

L1   ANSWER 3 OF 16  USPATFULL on STN
TI     Suppressor of HIV-1 replication and transcription

L1   ANSWER 4 OF 16  USPATFULL on STN
TI     Compounds which inhibit HIV replication

L1   ANSWER 5 OF 16  USPATFULL on STN
TI     Suppressor of HIV replication and transcription

L1   ANSWER 6 OF 16  USPATFULL on STN
TI     Methods for inhibition of membrane fusion-associated events, including
       respiratory syncytial virus transmission

L1   ANSWER 7 OF 16  USPATFULL on STN
TI     Methods for the inhibition of respiratory syncytial virus transmission

L1   ANSWER 8 OF 16  USPATFULL on STN
TI     Suppressor of HIV replication and transcription

L1   ANSWER 9 OF 16  USPATFULL on STN
TI     Anti-HIV compositions containing native and recombinant peptides

L1   ANSWER 10 OF 16  USPATFULL on STN
TI     Synthetic peptide inhibitors of HIV transmission

L1   ANSWER 11 OF 16  USPATFULL on STN
TI     cDNA cloning methods for the identification of a CD8+ T-lymphocytes
       suppressor factor capable of inhibiting HIV-1 replication

L1   ANSWER 12 OF 16  USPATFULL on STN
TI     Suppressor of HIV replication and transcription

L1   ANSWER 13 OF 16  USPATFULL on STN
TI     Supressor of HIV-1 replication and transcription

L1   ANSWER 14 OF 16  USPATFULL on STN
TI     Compounds which inhibit HIV replication

L1   ANSWER 15 OF 16  USPATFULL on STN
TI     Suppressor of HIV replication and transcription

L1   ANSWER 16 OF 16  USPATFULL on STN
TI     Synthetic peptide inhibitors of HIV transmission


=> d l1,cbib,ab,clm,1-16

L1   ANSWER 1 OF 16  USPATFULL on STN
2004:44245 Nucleic acids encoding DP-178 and other viral fusion inhibitor
    peptides useful for treating aids.
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Bolognesi, Dani Paul, Durham, NC, UNITED STATES
Matthews, Thomas James, Durham, NC, UNITED STATES
Wild, Carl T., Durham, NC, UNITED STATES
Duke University (U.S. corporation)
US 2004033235 A1 20040219
APPLICATION: US 2003-267682 A1 20030106 (10)
DOCUMENT TYPE: Utility; APPLICATION.

AB The present invention relates to peptides which exhibit potent anti-retroviral activity. The peptides of the invention comprise DP178 (SEQ ID:1) peptide corresponding to amino acids 638 to 673 of the HIV-1_{LAI} gp41 protein, and fragments, analogs and homologs of DP178. The invention further relates to the uses of such peptides as inhibitory of human and non-human retroviral, especially HIV, transmission to uninfected cells.

CLM What is claimed is:

1. An isolated peptide recognized by an ALLMOTI5, 107×178×4 or a PLZIP sequence search motif.

2. The peptide of claim 1 wherein the peptide corresponds to a peptide present in a virus.

3. The peptide of claim 2 in which the virus is HIV-1 or HIV-2.

4. The peptide of claim 2 in which the virus is a respiratory syncytial virus.

5. The peptide of claim 2 in which the virus is a human parainfluenza virus.

6. The peptide of claim 2 in which the virus is an influenza virus.

7. The peptide of claim 2 in which the virus is a hepatitis B virus.

8. The peptide of claim 2 wherein the virus is an Epstein-Barr virus.

9. A method for the inhibition of transmission of a virus to a cell, comprising contacting the cell with an effective concentration of a peptide recognized by an ALLMOTI5, 107x178x4 or a PLZIP sequence search motif for an effective period of time so that no infection of the cell by the virus occurs.

10. The method of claim 9 wherein the virus is HIV-1 or HIV-2.

11. The method of claim 9 wherein the virus is a respiratory syncytial virus.

12. The method of claim 9 wherein the virus is a human parainfluenza virus.

13. The method of claim 9 wherein the virus is an influenza virus.

14. The method of claim 9 in which the virus is a hepatitis B virus.

15. The method of claim 9 wherein the virus is an Epstein-Barr virus.

L1 ANSWER 2 OF 16 USPATFULL on STN
2003:258329 Compounds which inhibit HIV replication.
Wild, Carl T., Durham, NC, UNITED STATES
Matthews, Thomas J., Durham, NC, UNITED STATES
Bolognesi, Dani P., Durham, NC, UNITED STATES
Duke University (U.S. corporation)
US 2003181382 A1 20030925
APPLICATION: US 2003-414192 A1 20030415 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to human immunodeficiency virus (HIV) protein fragments which have antiviral activity, and particularly relates to HIV peptides derived from the HIV transmembrane glycoprotein (gp41) which inhibit HIV-induced cell-cell fusion. This invention further relates to methods for the inhibition of enveloped viral infection, and to methods that modulate biochemical processes which involve coiled coil peptide interactions.

CLM What is claimed is:

1. An isolated peptide comprising a DP-107 amino acid sequence listed in SEQ ID:1.

2. An isolated peptide ranging from about 14 to about 60 amino acids in length, capable of forming a heterodimer with the peptide of claim 1.

3. The peptide of claim 1 or 2 wherein the amino terminus of the peptide is acetylated.

4. The peptide of claim 1 or 2 wherein the carboxy terminus of the peptide is amidated.

5. An isolated multimer of the peptide of claim 1 or 2.

6. The multimer of claim 5 wherein the multimer is a tetramer.

7. The multimer of claim 5 wherein the multimer is a dimer consisting of two peptide monomers.

8. The dimer of claim 6 wherein the monomers of the dimer are covalently bound to one another.

9. A method for inhibiting HIV-induced cell fusion comprising contacting an HIV-infected cell with an effective amount of a peptide comprising a DP-107 amino acid sequence listed in SEQ ID:1 so that the cell fusion is inhibited.

10. The method of claim 9 wherein the HIV is HIV-1.

11. A method for inhibiting HIV-induced cell fusion comprising contacting an HIV-infected cell with an effective amount of a peptide comprising the peptide of claim 2 so that the cell fusion is inhibited.

12. The method of claim 11 wherein the HIV is HIV-1.

13. The method of claim 9 wherein the peptide is present as a multimer.

14. The method of claim 11 wherein the peptide is present as a multimer.

15. The method of claim 13 or 14 wherein the multimer is a dimer having two peptide monomers.

16. The method of claim 12 wherein the monomers are covalently bound to one another.

17. A method for testing compounds capable of inhibiting the ability of HIV to infect cells, comprising: (a) contacting a test compound to a multimer of a peptide comprising a DP-107 amino acid sequence listed in SEQ ID:1; and (b) detecting whether the test compound disrupts the multimer, the ability of the test compound to disrupt the multimer indicating the test compound is capable of inhibiting HIV infection of cells.

18. The method of claim 17 wherein the HIV is HIV-1.

19. The method of claim 17 wherein the multimer is a dimer or a tetramer.

20. The method of claim 17 wherein the contacting step is carried out in an aqueous solution.

21. A method for testing compounds capable of inhibiting the ability of HIV to infect cells, comprising: (a) contacting a test compound to a multimer of the peptide of claim 2; and (b) detecting whether the test compound disrupts the multimer, the ability of the test compound to disrupt the multimer indicating the test compound is capable of inhibiting HIV infection of cells.

22. The method of claim 21 wherein the HIV is HIV-1.

23. The method of claim 21 wherein the multimer is a dimer or a tetramer.

24. The method of claim 21 wherein the contacting step is carried out in an aqueous solution.

25. A method for inhibiting enveloped viral infection comprising contacting an uninfected cell with an effective amount of a peptide capable of contributing to the formation of a coiled coil peptide structure so that an enveloped virus is inhibited from infecting the uninfected cell.

26. The method of claim 25 wherein the enveloped virus is a retrovirus.

27. The method of claim 26 wherein the retrovirus is HIV-2, HTLV-I, or HTLV-II.

28. The method of claim 25 wherein the enveloped virus is an influenza virus.

29. The method of claim 25 wherein the enveloped virus is a respiratory syncytial virus.

29. A method for testing compounds capable of inhibiting the ability of an enveloped virus to infect cells, comprising: (a) contacting a test compound to a multimer of a peptide capable of contributing to the formation of a coiled coil peptide structure; and (b) detecting whether the test compound disrupts the multimer, the ability of the test compound to disrupt the multimer indicating the test compound is capable of inhibiting enveloped viral infection of cells.

30. The method of claim 29 wherein the enveloped virus is a retrovirus.

31. The method of claim 30 wherein the retrovirus is HIV-2, HTLV-I, or HTLV-II.

32. The method of claim 29 wherein the enveloped virus is an influenza virus.

33. The method of claim 29 wherein the multimer is a dimer or a tetramer.

34. The method of claim 29 wherein the contacting step is carried out in an aqueous solution.

L1 ANSWER 3 OF 16 USPATFULL on STN
2003:176279 Suppressor of HIV-1 replication and transcription.
Bolognesi, Dani P., Durham, NC, United States
Chen, Chin-Ho, Durham, NC, United States
Greenberg, Michael, Durham, NC, United States
Weinhold, Kent, Durham, NC, United States
Lacey, Simon F., Durham, NC, United States
Duke University, Durham, NC, United States (U.S. corporation)

US 6586174 B1 20030701
APPLICATION: US 1999-450141 19991129 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a bioactive molecule, herein referred to as the CD8+ suppressor molecule, that is produced by the CD8+ subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of viral transcription. The invention relates to isolation of clonal CD8+ cells lines that produce the antiviral activity and the development of an assay system for detection of the antiviral activity. The clonal cell lines and the assay system, described herein, may be utilized to purify, characterize and clone the CD8+ suppressor molecule. The CD8+ suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.
CLM What is claimed is:
1. A method for detecting a CD8+ suppressor molecule that has anti-HIV activity, comprising: (a) contacting a culture of cells with a sample containing a suppressor molecule, (i) wherein the culture of cells comprises cells genetically engineered to contain recombinant DNA expression vector in which a reporter gene is operatively associated with an HIV LTR promoter sequence, and (ii) wherein the sample containing the CD8+ suppressor molecule comprises culture supernatant from CD8+ cells or conditioned medium from CD8+ cells; and (b) measuring the inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with anti-HIV activity.

L1 ANSWER 4 OF 16 USPATFULL on STN
2003:148883 Compounds which inhibit HIV replication.
Wild, Carl T., Durham, NC, United States
Matthews, Thomas J., Durham, NC, United States
Bolognesi, Dani P., Durham, NC, United States
Duke University, Durham, NC, United States (U.S. corporation)
US 6573078 B1 20030603
APPLICATION: US 1995-464003 19950602 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to human immunodeficiency virus (HIV) protein fragments which have antiviral activity, and particularly relates to HIV peptides derived from the HIV transmembrane glycoprotein (gp41) which inhibit HIV-induced cell-cell fusion. This invention further relates to methods for the inhibition of enveloped viral infection, and to methods that modulate biochemical processes which involve coiled coil peptide interactions.
CLM What is claimed is:
1. An isolated peptide having the DP-107 amino acid sequence listed in SEQ ID NO:1.

2. The peptide of claim 1 wherein the amino terminus of the peptide is acetylated.

3. The peptide of claim 1 wherein the carboxy terminus of the peptide is amidated.

4. An isolated multimer of the peptide of claim 1.

5. The multimer of claim 4 wherein the multimer is a tetramer.

6. The multimer of claim 4 wherein the multimer is a dimer consisting of two peptide monomers.

7. The dimer of claim 6 wherein the monomers of the dimer are covalently bound to one another.

8. A method for inhibiting fusion of HIV to a cell comprising contacting the cell with a peptide in an effective amount to inhibit cell fusion, wherein the peptide is the peptide of claim 1.

9. The method of claim 8 wherein the HIV is HIV-1.

10. The method of claim 8 wherein the peptide is present as a multimer.

11. The method of claim 10 wherein the multimer is a dimer having two peptide monomers.

12. A method for inhibiting transmission of HIV to a cell comprising contacting the cell with a peptide in an effective amount to inhibit infection of the cell, wherein the peptide is the peptide of claim 1.

13. The method of claim 12 wherein the HIV is HIV-1.

14. The method of claim 12 wherein the peptide is present as a multimer.

15. The method of claim 14 wherein the multimer is a dimer having two peptide monomers.

16. The method of claim 15 wherein the monomers are covalently bound to one another.

17. An isolated peptide having the DP-125 amino acid sequence listed in SEQ ID NO:3.

18. An isolated peptide having the DP-127 amino acid sequence listed in SEQ ID NO:8.

19. An isolated peptide having the DP-129 amino acid sequence listed in SEQ ID NO:9.

20. An isolated peptide having the DP-130 amino acid sequence listed in SEQ ID NO:10.

21. An isolated peptide having the DP-137 amino acid sequence listed in SEQ D NO:12.

22. An isolated multimer of a peptide selected from the group consisting of DP-125 (SEQ ID NO:3), DP-127 (SEQ ID NO:8), DP-129 (SEQ ID NO:9), DP-130 (SEQ ID NO:10), and DP-137 (SEQ ID NO:12).

23. The multimer of claim 22, wherein the multimer comprises a dimer having two peptide monomers.

24. The multimer of claim 22, wherein the multimer comprises a tetramer.

25. A method for inhibiting fusion of HIV to a cell comprising contacting the cell with a peptide in an effective amount to inhibit cell fusion, wherein the peptide is selected from the group consisting of DP-125 (SEQ ID NO:3), DP-127 (SEQ ID NO:8), DP-129 (SEQ ID NO:9), DP-130 (SEQ ID NO:10), and DP-137 (SEQ ID NO:12).

26. A method for inhibiting transmission of HIV to a cell comprising contacting the cell with a peptide in an effective amount to inhibit infection of the cell, wherein the peptide is selected from the group consisting of DP-125 (SEQ ID NO:3), DP-127 (SEQ ID NO:8), DP-129 (SEQ ID NO:9), DP-130 (SEQ ID NO:10), and DP-137 (SEQ ID NO:12).

L1 ANSWER 5 OF 16 USPATFULL on STN
2003:60103 Suppressor of HIV replication and transcription.
Bolognesi, Dani P., Durham, NC, United States
Greenberg, Michael L., Durham, NC, United States

Lacey, Simon F., Azusa, CA, United States
Tomaras, Georgia D., Durham, NC, United States
Weinhold, Kent J., Durham, NC, United States
Duke University, Durham, NC, United States (U.S. corporation)
US 6528308 B1 20030304
APPLICATION: US 2000-527320 20000316 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a bioactive molecule, herein referred to as the CD8+ suppressor molecule, that is produced by the CD8+ subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of viral transcription. The invention relates to isolation of CD8+ cell lines and cell clones that produce that antiviral activity and to the development of assay systems for detection of the antiviral activity. The cell lines, cell clones and assay systems, described herein, may be utilized, e.g., to purify, characterize and clone the CD8+ suppressor molecule. The CD8+ suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.
CLM What is claimed is:
1. A permanently established lymphocyte cell line identified as DU.JR-HVS (ATCC Accession No. PTA-1551).

2. A permanently established lymphocyte cell line identified as DU.HS-HVS (ATCC Accession No. PTA-1552).

L1 ANSWER 6 OF 16 USPATFULL on STN
2002:297296 Methods for inhibition of membrane fusion-associated events, including respiratory syncytial virus transmission.
Bolognesi, Dani Paul, Durham, NC, United States
Matthews, Thomas James, Durham, NC, United States
Wild, Carl T., Durham, NC, United States
Barney, Shawn O'Lin, Cary, NC, United States
Lambert, Dennis Michael, Cary, NC, United States
Petteway, Stephen Robert, Cary, NC, United States
Langlois, Alphonse J., Durham, NC, United States
Trimeris, Inc., Durham, NC, United States (U.S. corporation)
US 6479055 B1 20021112
APPLICATION: US 1995-470896 19950606 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to peptides which exhibit potent anti-viral activity. In particular, the invention relates to methods of using such peptides as inhibitory of respiratory syncytial virus ("RSV") transmission to uninfected cells. The peptides used in the methods of the invention are homologs of the DP-178 and DP-107 peptides, peptides corresponding to amino acid residues 638 to 673, and to amino acid residues 558 to 595, respectively, of the HIV-1_{LAI} transmembrane protein (TM) gp41.
CLM What is claimed is:
1. A method for inhibiting transmission of a respiratory syncytial virus to a cell, said method comprising contacting said cell with an effective concentration of an isolated peptide for an effective period of time; wherein said isolated peptide comprises an amino acid sequence of a respiratory syncytial virus protein; wherein said isolated peptide is identified by one or more of an ALLMOTI5, 107x178'4 and PLZIP sequence search motifs; and wherein fusion of the virus to the cell is inhibited.

2. A method for inhibiting transmission of a respiratory syncytial virus to a cell, said method comprising contacting said cell with an effective concentration of an isolated peptide; wherein said isolated peptide has the formula: X-TSVITIELSNIKENKCNGTDAKVKLIKQELDKYKN-Z; X-SVITIELSNIKENKCNGTDAKVKLIKQELDKYKNA-Z; X-VITIELSNIKENKCNGTDAKVKLIKQELD

KYKNAV-Z; X-VAVSKVLHLEGEVNKIALLSTNKAVVSLSNGVS-Z; X-AVSKVLHLEGEVNKIALLSTNKAVVSLSNGVSV-Z; X-VSKVLHLEGEVNKIALLSTNKAVVSLSNGVSVL-Z; X-SKVLHLEGEVNKIALLSTNKAVVSLSNGVSVLT-Z; X-KVLHLEGEVNKIALLSTNKAVVSLSNGVSVLTS-Z; X-LEGEVNKIALLSTNKAVVSLSNGVSVLTSKVLD-Z; X-GEVNKIALLSTNKAVVSLSNGVSVLTSKVLDLK-Z; X-EVNKIALLSTNKAVVSLSNGVSVLTSKVLDLKN-Z; X-VNKIALLSTNKAVVSLSNGVSVLTSKVLDLKNY-Z; X-NKIALLSTNKAVVSLSNGVSVLTSKVLDLKNYI-Z; X-KIALLSTNKAVVSLSNGVSVLTSKVLDLKNYID-Z; X-IALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDK-Z; X-ALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQ-Z; X-VAVSKVLHLEGEVNKIALLSTNKAVVSLSNGVS-Z; X-AVSKVLHLEGEVNKIALLSTANKVVSLSNGVSV-Z; X-VSKVLHLEGEVNKIALLSTNKAVVSLSNGVSVL-Z; X-SKVLHLEGEVNKIALLSTNKAVVSLSNGVSVLT-Z; X-KVLHLEGEVNKIALLSTNKAVVSLSNGVSVLTS-Z; X-LEGEVNKIALLSTNKAVVSLSNGVSVLTSKVLD-Z; X-GEVNKIALLSTNKAVVSLSNGVSVLTSKVLDLK-Z; X-EVNKIALLSTNKAVVSLSNGVSVLTSKVLDLKN-Z; X-VNKIALLSTNKAVVSLSNGVSVLTSKVLDLKNY-Z: X-NKIALLSTNKAVVSLSNGVSVLTSKVLDLKNYI-Z; X-KIALLSTNKAVVSLSNGVSVLTSKLDLKCNYID-Z; or X-IALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDK-Z, (SEQ ID NOS:210-237, respectively); in which: amino acid residues are presented by the single-letter code; X comprises the amino group of said peptide, an acetyl group, a 9-fluorenylmethoxy carbonyl group, a hydrophobic group, or a macromolecule carrier group; Z comprises the carboxyl group of said peptide, an amido group, a hydrophobic group, or a macromolecular carrier group; and wherein fusion of said virus to said cell is inhibited.

3. The method of claim 2, wherein said peptide has the formula: X-TSVITIELSNIKENKCNGTDAKVKLIKQELDKYKN-Z (SEQ ID NO:210).

4. The method of claim 2, wherein said peptide has the formula: X-SVITIELSNIKENKCNGTDAKVKLIKQELDKYKNA-Z (SEQ ID NO:211).

5. The method of claim 2, wherein said peptide has the formula: X-VITIELSNIKENKCNGTDAKVKLIKQELDKYKNAV-Z (SEQ ID NO:212).

6. The method of claim 2, wherein said peptide has the formula: X-VAVSKVLHLEGEVNKIALLSTNKAVVSLSNGVS-Z (SEQ ID NO:213).

7. The method of claim 2, wherein said peptide has the formula: X-AVSKVLHLEGEVNKIALLSTNKAVVSLSNGVSV-Z (SEQ ID NO:214).

8. The method of claim 2, wherein said peptide has the formula: X-VSKVLHLEGEVNKIALLSTNKAVVSLSNGVSVL-Z (SEQ ID NO:215).

9. The method of claim 2, wherein said peptide has the formula: X-SKVLHLEGEVNKIALLSTNKAVVSLSNGVSVLT-Z (SEQ ID NO:216).

10. The method of claim 2, wherein said peptide has the formula: X-KVLHLEGEVNKIALLSTNKAVVSLSNGVSVLTS-Z (SEQ ID NO:217).

11. The method of claim 2, wherein said peptide has the formula: X-LEGEVNKIALLSTNKAVVSLSNGVSVLTSKVLD-Z (SEQ ID NO:218).

12. The method of claim 2, wherein said peptide has the formula: X-GEVNKIALLSTNKAVVSLSNGVSVLTSKVLDLK-Z (SEQ ID NO:219).

13. The method of claim 2, wherein said peptide has the formula: X-EVNKIALLSTNKAVVSLSNGVSVLTSKVLDLKN-Z (SEQ ID NO:220).

14. The method of claim 2, wherein said peptide has the formula: X-VNKIALLSTNKAVVSLSNGVSVLTSKVLDLKNY-Z (SEQ ID NO:221).

15. The method of claim 2, wherein said peptide has the formula: X-NKIALLSTNKAVVSLSNGVSVLTSKVLDLKNYI-Z (SEQ ID NO:222).

16. The method of claim 2, wherein said peptide has the formula: X-KIALLSTNKAVVSLSNGVSVLTSKVLDLKNYID-Z (SEQ ID NO:223).

17. The method of claim 2, wherein said peptide has the formula: X-IALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDK-Z (SEQ ID NO:224).

18. The method of claim 2, wherein said peptide has the formula: X-ALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQ-Z (SEQ ID NO:225).

19. The method of claim 2, wherein said peptide has the formula: X-VAVSKVLHLEGEVNKIALLSTNKAVVSLSNGVS-Z (SEQ ID NO:226).

20. The method of claim 2, wherein said peptide has the formula: X-AVSKVLHLEGEVNKIALLSTNKAVVSLSNGVSV-Z (SEQ ID NO:227).

21. The method of claim 2, wherein said peptide has the formula: X-VSKVLHLEGEVNKIALLSTNKAVVSLSNGVSVL-Z (SEQ ID NO:228).

22. The method of claim 2, wherein said peptide has the formula: X-SKVLHLEGEVNKIALLSTNKAVVSLSNGVSVLT-Z (SEQ ID NO:229).

23. The method of claim 2, wherein said peptide has the formula: X-KVLHLEGEVNKIALLSTNKAVVSLSNGVSVLTS-Z (SEQ ID NO:230).

24. The method of claim 2, wherein said peptide has the formula: X-LEGEVNKIALLSTNKAVVSLSNGVSVLTSKVLD-Z (SEQ ID NO:231).

25. The method of claim 2, wherein said peptide has the formula: X-GEVNKIALLSTNKAVVSLSNGVSVLTSKVLDLK-Z (SEQ ID NO:232).

26. The method of claim 2, wherein said peptide has the formula: X-EVNKIALLSTNKAVVSLSNGVSVLTSKVLDLKN-Z (SEQ ID NO:233).

27. The method of claim 2, wherein said peptide has the formula: X-VNKIALLSTNKAVVSLSNGVSVLTSKVLDLKNY-Z (SEQ ID NO:234).

28. The method of claim 2, wherein said peptide has the formula: X-NKIALLSTNKAVVSLSNGVSVLTSKVLDLKNYI-Z (SEQ ID NO:235).

29. The method of claim 2, wherein said peptide has the formula: X-KIALLSTNKAVVSLSNGVSVLTSKVLDLKNYID-Z (SEQ ID NO:236).

30. The method of claim 2, wherein said peptide has the formula: X-IALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDK-Z (SEQ ID NO:237).

31. The method of claim 1, wherein said peptide is 28 amino acid residues in length and is identified by the ALLMOTI5 motif.

32. The method of claim 1, wherein said peptide is 35 amino acid residues in length and is identified by the ALLMOTI5 motif.

33. The method of claim 1, wherein said peptide is 28 amino acid residues in length and is identified by the 107x178x4 motif.

34. The method of claim 1, wherein said peptide is 35 amino acid residues in length and is identified by the 107x178x4 motif.

35. The method of claim 1, wherein said peptide is identified by a PLZIP motif.

36. The method of claim 2 wherein X is a macromolecular carrier group.

37. The method of claim 36 wherein the macromolecular carrier group X is a peptide group.

38. The method of claim 37 wherein said peptide group is 2 to 50 respiratory syncytial virus protein amino acid residues amino to said isolated peptide.

39. The method of claim 2 wherein Z is a macromolecular carrier group.

40. The method of claim 39 wherein the macromolecular carrier group Z is a peptide group.

41. The method of claim 40 wherein said peptide group is about 2 to about 50 respiratory syncytial virus protein amino acid residues carboxy to said isolated peptide.

42. The method of claim 41 wherein X is a macromolecular carrier group, said macromolecular carrier group X being a peptide group of 2 to 50 respiratory syncytial virus protein amino acid residues amino to said isolated peptide.

43. A method for inhibiting transmission of a respiratory syncytial virus to a cell, said method comprising contacting the cell with an effective concentration of an isolated peptide for an effective period of time; wherein said peptide consists of an amino acid sequence of a respiratory syncytial virus protein; wherein said peptide is identified by one or more of an ALLMOTI5, 107x178x4 and PLZIP sequence search motifs; and wherein fusion of the virus to the cell in inhibited.

44. A method for inhibiting transmission of a respiratory syncytial virus to a cell, said method comprising contacting the cell with an effective concentration of an isolated peptide for an effective period of time; wherein said peptide consists of an amino acid sequence of a respiratory syncytial virus protein; wherein said peptide is identified by one or more of an ALLMOTI5, 107x178x4 and PLZIP sequence search motifs; wherein said peptide further comprises an amino terminal X, and a carboxy terminal Z in which X comprises the amino group of said peptide, an acetyl group, a 9-fluorenylmethoxy- carbonyl group, a hydrophobic group or a macromolecular carrier group, and Z comprises the carboxyl group of said peptide, an amido group, a hydrophobic group or a macromolecular carrier group; and wherein fusion of the virus to the cell in inhibited.

L1 ANSWER 7 OF 16 USPATFULL on STN
2002:217016 Methods for the inhibition of respiratory syncytial virus transmission.
Bolognesi, Dani Paul, Durham, NC, United States
Matthews, Thomas James, Durham, NC, United States
Wild, Carl T., Durham, NC, United States
Barney, Shawn O'Lin, Cary, NC, United States
Lambert, Dennis Michael, Cary, NC, United States
Petteway, Jr., Stephen Robert, Cary, NC, United States
Trimeris, Inc., Durham, NC, United States (U.S. corporation)
US 6440656 B1 20020827
APPLICATION: US 1994-255208 19940607 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Fusion of the viral envelope, or infected cell membranes with uninfected cell membranes, is an essential step in the viral life cycle. Recent studies involving the human immunodeficiency virus type 1 (HIV-1) demonstrated that synthetic peptides (designated DP-107 and DP-178) derived from potential helical regions of the transmembrane (TM) protein, gp41, were potent inhibitors of viral fusion and infection. A computerized antiviral searching technology (C.A.S.T.) that detects related structural motifs (e.g., ALLMOTI5, 107x178x4, and PLZIP) in other viral proteins was employed to identify similar regions in the respiratory syncytial virus (RSV). Several conserved heptad repeat domains that are predicted to form coiled-coil structures with antiviral activity were identified in the RSV genome. Synthetic peptides of 16 to 39 amino acids derived from these regions were prepared and their antiviral activities assessed in a suitable in vitro screening

assay. These peptides proved to be potent inhibitors of RSV fusion. Based upon their structural and functional equivalence to the known HIV-1 inhibitors DP-107 and DP-178, these peptides should provide a novel approach to the development of targeted therapies for the treatment of RSV infections.

What is claimed is:

1. A method for the inhibition of transmission of a respiratory syncytial virus to a cell, comprising contacting the cell with an effective concentration of an isolated peptide consisting of an amino acid sequence of a 16 to 39 amino acid residue region of a respiratory syncytial virus protein for an effective period of time, wherein: (a) said region is recognized by an ALLMOTI5, 107×178×4, or PLZIP sequence search motif; (b) said peptide further comprises an amino terminal X, and a carboxy terminal Z in which: X comprises an amino group, an acetyl group, a 9-fluorenylmethoxy-carbonyl group, a hydrophobic group, or a macromolecular carrier group; and Z comprises a carboxyl group, an amido group, a hydrophobic group, or a macromolecular carrier group; and (c) fusion of the virus to the cell is inhibited.

2. A method for the inhibition of transmission of a respiratory syncytial virus to a cell, comprising contacting the cell with an effective concentration of a peptide for an effective period of time, wherein the peptide has the formula: X-FYDPLVFPSDEFDASISQVNEKINQSLAFIRKS DE-Z (SEQ ID NO:68); X-DPLVFPSDEFDASISQVNEKINQSLAFIRKSDELL-Z (SEQ ID NO:101); X-YDPLVFPSDEFDASISQVNEKINQSLAFIRKSDEL-Z (SEQ ID NO:103); X-LVFPSDEFDASISQVNEKINQSLAFIRKSDELLHN-Z (SEQ ID NO:104); X-VFPSDEFDASISQVNEKINQSLAFIRKSDELLHNV-Z (SEQ ID NO:105); X-FPSDEFDASISQVNEKINQSLAFIRKSDELLHNVN-Z (SEQ ID NO:106); X-PSDEFDASISQVNEKINQSLAFIRKSDELLHNVNA-Z (SEQ ID NO:107); X-SDEFDASISQVNEKINQSLAFIRKSDELLHNVNAG-Z (SEQ ID NO:108); X-DEFDASISQVNEKINQSLAFIRKSDELLHNVNAGK-Z (SEQ ID NO:109); X-FDASISQVNEKINQSLAFIRKSDELLHNVNAGKST-Z (SEQ ID NO:110); or X-DASISQVNEKINQSLAFIRKSDELLHNVNAGKSTT-Z (SEQ ID NO:111) in which: amino acid residues are presented by the single-letter code; X comprises an amino group, an acetyl group, a 9-fluoromethyoxymethyl-carbonyl group, a hydrophobic group, or a macromolecular carrier group; Z comprises a carboxyl group, an amido group, a hydrophobic group, or a macromolecular carrier group; and wherein fusion of the virus to the cell is inhibited.

3. The method of claim 2, wherein the peptide has the formula: X-DPLVFPSDEFDASISQVNEKINQSLAFIRKSDELL-Z (SEQ ID NO. 101).

4. The method of claim 2, wherein the peptide has the formula: X-YDPLVFPSDEFDASISQVNEKINQSLAFIRKSDEL-Z (SEQ ID NO. 103).

5. The method of claim 2, wherein the peptide has the formula: X-LVFPSDEFDASISQVNEKINQSLAFIRKSDELLHN-Z (SEQ ID NO. 104).

6. The method of claim 2, wherein the peptide has the formula: X-VFPSDEFDASISQVNEKINQSLAFIRKSDELLHNV-Z (SEQ ID NO. 105).

7. The method of claim 2, wherein the peptide has the formula: X-FPSDEFD ASISQVNEKINQSLAFIRKSDELLHNVN-Z (SEQ ID NO. 106).

8. The method of claim 2, wherein the peptide has the formula: X-PSDEFDASISQVNEKINQSLAFIRKSDELLHNVNA-Z (SEQ ID NO. 107).

9. The method of claim 2, wherein the peptide has the formula: X-SDEFDASISQVNEKINQSLAFIRKSDELLHNVNAG-Z (SEQ ID NO. 108).

10. The method of claim 2, wherein the peptide has the formula: X-DEFDASISQVNEKINQSLAFIRKSDELLHNVNAGK-Z (SEQ ID NO. 109).

11. The method of claim 2, wherein the peptide has the formula: X-FDASISQVNEKINQSLAFIRKSDELLHNVNAGKST-Z (SEQ ID NO. 110).

12. The method of claim 2, wherein the peptide has the formula: X-DASISQVNEKINQSLAFIRKSDELLHNVNAGKSTT-Z (SEQ ID NO. 111).

13. The method of claim 2, wherein the peptide has the formula: X-FYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDE-Z (SEQ ID NO. 68).

ANSWER 8 OF 16 USPATFULL on STN
02:191473 Suppressor of HIV replication and transcription.
Bolognesi, Dani P., Durham, NC, UNITED STATES
Greenberg, Michael L., Durham, NC, UNITED STATES
Lacey, Simon F., Azusa, CA, UNITED STATES
Tomaras, Georgia D., Durham, NC, UNITED STATES
Weinhold, Kent J., Durham, NC, UNITED STATES
Duke University (U.S. corporation)
US 2002102538 A1 20020801
APPLICATION: US 2002-71349 A1 20020206 (10)
DOCUMENT TYPE: Utility; APPLICATION.
AS INDEXING IS AVAILABLE FOR THIS PATENT.
The present invention relates to a bioactive molecule, herein referred to as the CD8+ suppressor molecule, that is produced by the CD8+ subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of viral transcription. The invention relates to isolation of CD8+ cell lines and cell clones that produce that antiviral activity and to the development of assay systems for detection of the antiviral activity. The cell lines, cell clones and assay systems, described herein, may be utilized, e.g., to purify, characterize and clone the CD8+ suppressor molecule. The CD8+ suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.
LM What is claimed is:
1. A method for detecting a CD8+ suppressor molecule that has anti-HIV-1 activity, the method comprising: (a) contacting a host cell with a replication deficient HIV pseudotyped virus comprising a reporter gene operatively associated with an HIV promoter; (b) contacting the host cell with: (i) a sample comprising enriched CD8+ cells; or (ii) a sample comprising a cell culture of CD8+ cells; or (iii) an extract or media component from (i) or (ii); and (c) measuring reporter gene activity, wherein inhibition of reporter gene activity indicates anti-HIV-1 activity.

2. The method of claim 1, wherein the reporter gene is expressed during early proviral gene expression.

3. The method of claim 2, wherein the reporter gene is expressed in place of an early proviral gene.

4. The method of claim 3, wherein the early proviral gene is a nef gene.

5. The method of claim 1, wherein the pseudotyped virus is an env deficient pseudotyped virus.

6. The method of claim 5, wherein the pseudotyped virus is produced by a method comprising co-transfecting DNA for the pseudotyped virus with a vector that encodes a viral envelope protein.

7. The method of claim 6, wherein the viral envelope protein is an HIV Env protein.

8. The method of claim 6, wherein the viral envelope protein is a non-HIV viral envelope protein.

9. The method of claim 1, wherein the reporter gene is a luciferase gene, a chloramphenicol acetyltransferase gene, a growth hormone gene, or a fluorescent protein gene.

10. The method of claim 9, wherein the reporter gene is a luciferase gene.

11. A method for detecting a CD8+ suppressor molecule that has anti-HIV-1 activity, said method comprising: (a) contacting a host cell with an env deficient HIV pseudotyped virus comprising a reporter gene substituted for an HIV nef gene such that said reporter gene is expressed in place of the HIV nef gene; (b) contacting the host cell with: (i) a sample comprising enriched CD8+ cells; or (ii) a sample comprising a cell culture of CD8+ cells; or (iii) an extract or media component from (i) or (ii); and (c) measuring reporter gene activity, wherein inhibition of reporter gene activity indicates anti-HIV-1 activity.

12. The method of claim 11, wherein the reporter gene is a luciferase gene, a chloramphenicol acetyltransferase gene, a growth hormone gene, or a fluorescent protein gene.

13. The method of claim 12, wherein the reporter gene is a luciferase gene.

14. A diagnostic assay for monitoring clinical progression of HIV infection, the diagnostic assay comprising: (a) contacting a host cell with a replication deficient HIV pseudotyped virus comprising a reporter gene operatively associated with an HIV promoter; (b) contacting the host cell with samples from an HIV infected individual, wherein the samples are collected from the individual at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals, wherein an increase in reporter gene activity indicates progression of HIV infection.

15. The method of claim 14, wherein the reporter gene is expressed during early proviral gene expression.

16. The method of claim 15, wherein the reporter gene is expressed in place of an early proviral gene.

17. The method of claim 16, wherein the early proviral gene is a nef gene.

18. The method of claim 16, wherein the pseudotyped virus is an env deficient pseudotyped virus.

19. The method of claim 18, wherein the pseudotyped virus is produced by a method comprising co-transfecting DNA for said pseudotyped virus with a vector that encodes a viral envelope protein.

20. The method of claim 19, wherein the viral envelope protein is an HIV Env protein.

21. The method of claim 19, wherein the viral envelope protein is a non-HIV viral envelope protein.

22. The method of claim 14, wherein the reporter gene is a chloramphenicol acetyltransferase gene, a luciferase gene, a growth hormone gene, or a fluorescent protein gene.

23. The method of claim 22, wherein the reporter gene is a luciferase gene.

24. A diagnostic assay for monitoring clinical progression of HIV infection, the diagnostic assay comprising: (a) contacting a host cell with an env deficient HIV pseudotyped virus comprising a reporter gene substituted for an HIV nef gene such that said reporter gene is expressed in place of the HIV nef gene; (b) contacting the host cell

with samples from an HIV infection individual, wherein the samples are collected from the individual at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals, wherein an increase in reporter gene activity indicates progression of HIV infection.

25. The method of claim 24, wherein the reporter gene is a chloramphenicol acetyltransferase gene, a luciferase gene, a growth hormone gene, or a fluorescent protein gene.

26. The method of claim 25, wherein the reporter gene is a luciferase gene.

27. A method for detecting a compound that suppresses HIV-1 replication, the method comprising: (a) contacting a host cell with a replication deficient HIV pseudotyped virus comprising a reporter gene operatively associated with an HIV promoter; (b) contacting the host cell with the compound at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals, wherein inhibition of reporter gene activity at one or more time intervals indicates that the compound suppresses HIV-1 replication.

28. The method of claim 27, wherein the reporter gene is expressed during early proviral gene expression.

29. The method of claim 28, wherein the reporter gene is expressed in place of an early proviral gene.

30. The method of claim 29, wherein the early proviral gene is a nef gene.

31. The method of claim 27, wherein the pseudotyped virus in an env deficient pseudotyped virus.

32. The method of claim 31, wherein the pseudotyped virus is produced by a method comprising co-transfecting DNA for the pseudotyped virus with a vector that encodes a viral envelope protein.

33. The method of claim 32, wherein the viral envelope protein is an HIV Env protein.

34. The method of claim 32, wherein the viral envelope protein is a non-HIV envelope protein.

35. The method of claim 27, wherein suppression of HIV-1 is at a stage of viral entry.

36. The method of claim 35 further comprising the steps of: (a) contacting a different host cell with the HIV pseudotyped virus; (b) contacting the different host cell with a viral entry inhibitor at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals; wherein the time intervals at which reporter gene activity is inhibited correspond to time intervals of viral entry.

37. The method of claim 36, wherein the viral entry inhibitor is an anti-fusion peptide.

38. The method of claim 37, wherein the anti-fusion peptide is DP107, DP178, T1249 or T649.

39. The method of claim 36, wherein the viral entry inhibitor is an antibody that disrupts the interaction between a CD4+ cell surface receptor and a viral envelope protein.

40. The method of claim 39, wherein the antibody is a monoclonal antibody that specifically binds to the CD4+ receptor.

41. The method of claim 27, wherein suppression of HIV-1 is at a stage of reverse transcription.

42. The method of claim 41 further comprising the steps of: (a) contacting a different host cell with the HIV pseudotyped virus; (b) contacting the different host cell with a reverse transcription inhibitor at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals; wherein the time intervals at which reporter gene activity is inhibited correspond to time intervals of reverse transcription.

43. The method of claim 42, wherein the reverse transcription inhibitor is a non-nucleoside reverse transcriptase inhibitor.

44. The method of claim 43, wherein the reverse transcriptase inhibitor is nevirapine.

45. The method of claim 27, wherein suppression of HIV-1 is at a stage of early virus gene expression.

46. The method of claim 45 further comprising the steps of: (a) contacting a different host cell with the HIV pseudotyped virus; (b) contacting the different host cell with an inhibitor of early virus gene expression at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals, wherein the time intervals at which reporter gene activity is inhibited correspond to time intervals of early virus gene expression.

47. The method of claim 46, wherein the inhibitor of early virus gene expression is a Tat inhibitor.

ANSWER 9 OF 16 USPATFULL on STN
01:157801 Anti-HIV compositions containing native and recombinant peptides.
Fischinger, Peter J., Baltimore, MD, United States
Wong-Stall, Flossie, Rockville, MD, United States
Gallo, Robert C., Bethesda, MD, United States
Matthews, Thomas J., Durham, NC, United States
Bolognesi, Dani P., Durham, NC, United States
Robey, Gerard W., Third Lake, IL, United States
Krohn, Kai, Tampere, Finland
Ranki, Annamarie, Tampere, Finland
The United States of America as represented by the Secretary of the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 6290963 B1 20010918
APPLICATION: US 1989-314664 19890223 (7)
DOCUMENT TYPE: Utility; GRANTED.
S INDEXING IS AVAILABLE FOR THIS PATENT.
Native and recombinant peptides which elicit anti-HIV immune response are provided.
M What is claimed is:
1. An HIV polypeptide PB1, consisting of an amino acid sequence as shown in FIG. 5.

2. A composition comprising an immunogenic amount of the polypeptide of claim 1 and a pharmaceutically acceptable carrier.

3. A method of eliciting an immune response, the method comprising administering to a responsive host an immunogenic amount of the polypeptide of claim 1 to induce an anti-HIV immune response.

4. A method of producing, in a mammal, antibodies that neutralize HIV, the method comprising administering to said mammal an amount of the polypeptide of claim 1 sufficient to effect said production.

5. An HIV polypeptide PB1 comprising the amino acid sequence encoded by a Pvull to BgIII fragment of an HIV env gene.

6. An immunogenic composition comprising a polypeptide having the antigenic properties of a polypeptide according to claim 5.

7. An immunogenic composition comprising a hybrid PB1 polypeptide having the amino-acid sequcnce of a PB1 polypeptide from different HIV isolates.

8. An immunogenic composition according to claim 7, wherein the HIV isolates are HIV_B, HIV_{MN}, and HIV_{RF}.

ANSWER 10 OF 16 USPATFULL on STN
000:138503 Synthetic peptide inhibitors of HIV transmission.
Bolognesi, Dani Paul, Durham, NC, United States
Matthews, Thomas James, Durham, NC, United States
Wild, Carl T., Durham, NC, United States
Duke University, Durham, NC, United States (U.S. corporation)
US 6133418 20001017
APPLICATION: US 1995-554616 19951106 (8)
DOCUMENT TYPE: Utility; Granted.
AS INDEXING IS AVAILABLE FOR THIS PATENT.
B The present invention relates to peptides which exhibit potent anti-retroviral activity. The peptides of the invention comprise DP-178 (SEQ ID:1) ptide corresponding to amino acids 638 to 673 of the HIV-1_{LAI} gp41 protein, and fragments, analogs and homologs of DP-178. The invention further relates to the uses of such peptides as inhibitory of human and non-human retroviral, especially HIV, transmission to uninfected cells.
LM What is claimed is:
1. A peptide having the formula X-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z (SEQ ID NO:1) in which amino acid residues are presented by the single-letter code wherein: X is an amino group, an acetyl group, a 9-fluorenylmethoxy-carbonyl group, or a hydrophobic group; and Z is a carboxyl group, an amido group, or a hydrophobic group.

2. A peptide having the formula X-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z (SEQ ID NO: 1) in which amino acid residues are presented by the single-letter code wherein: X is an acetyl group; and Z is an amido group.

3. A pharmaceutical composition comprising an effective amount of the peptide of claim 2 and a pharmaceutically acceptable carrier or excipient.

1 ANSWER 11 OF 16 USPATFULL on STN
999:155438 cDNA cloning methods for the identification of a CD8+ T-lymphocytes suppressor factor capable of inhibiting HIV-1 replication.
Bolognesi, Dani P., Durham, NC, United States
Chen, Chin-Ho, Durham, NC, United States
Greenberg, Michael, Durham, NC, United States
Weinhold, Kent, Durham, NC, United States
Lacey, Simon F., Durham, NC, United States
Duke University Medical Center, Durham, NC, United States (U.S. corporation)
US 5994054 19991130
APPLICATION: US 1995-486810 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
AS INDEXING IS AVAILABLE FOR THIS PATENT.
B The present invention relates to a bioactive molecule, herein referred to as the CD8+ suppressor molecule, that is produced by the CD8+ subset of human T-lymphocytes and suppresses type-1 human

immunodeficiency virus (HIV-1) replication through inhibition of viral transcription. The invention relates to isolation of clonal CD8+ cells lines that produce the antiviral activity and the development of an assay system for detection of the antiviral activity. The clonal cell lines and the assay system, described herein, may be utilized to purify, characterize and clone the CD8+ suppressor molecule. The CD8+ suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.

CLM What is claimed is:

1. A method for isolating a recombinant cDNA clone encoding a CD8+ suppressor molecule that inhibits HIV replication comprising: (a) constructing a cDNA expression library using mRNA prepared from CD8+ T-lymphocytes that express the CD8+ suppressor molecule; and (b) screening the cDNA products using a method comprising: (i) culturing a host cell line transfected with a recombinant DNA expression vector in which a reporter gene is operatively associated with an HIV LTR promoter sequence; (ii) contacting the cell line with a sample comprising enriched CD8+ cells or cell cultures of CD8+ cells; and (iii) measuring the inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with anti-HIV activity.

2. A method for isolating a recombinant cDNA clone encoding a CD8+ suppressor molecule that inhibits HIV replication comprising: (a) constructing a cDNA expression library using mRNA prepared from CD8+ T-lymphocytes that express the CD8+ suppressor molecule; (b) enriching the cDNA library by eliminating clones that hybridize to cDNAs prepared from mRNA of lymphocytes that do not express the CD8+ suppressor molecule; and (c) screening the enriched cDNA products using a method comprising: (i) culturing a host cell line transfected with a recombinant DNA expression vector in which a reporter gene is operatively associated with an HIV LTR promoter sequence; (ii) contacting the cell line with a sample comprising enriched CD8+ cells or cell cultures of CD8+ cells; and (iii) measuring the inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with anti-HIV activity.

3. The method of claim 1 in which the CD8+ lymphocyte is the lymphocyte clone deposited with the American Type Culture Collection and assigned accession number CRL 11310.

4. The method of claim 2 in which the CD8+ lymphocyte is the lymphocyte clone deposited with the American Type Culture Collection and assigned accession number CRL 11310.

5. The method of claim 2 in which the lymphocyte that does not express the CD8+ suppressor molecule is the lymphocyte cell clone deposited with the American Type Culture Collection and assigned accession number CRL 11309.

L1 ANSWER 12 OF 16 USPATFULL on STN

1999:7476 Suppressor of HIV replication and transcription.

Bolognesi, Dani P., Durham, NC, United States
Chen, Chin-Ho, Durham, NC, United States
Greenberg, Michael, Durham, NC, United States
Weinhold, Kent, Durham, NC, United States
Lacey, Simon F., Durham, NC, United States
Duke University Medical Center, Durham, NC, United States (U.S. corporation)

US 5861490 19990119
APPLICATION: US 1995-471430 19950606 (8)
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a bioactive molecule, herein referred to as the CD8+ suppressor molecule, that is produced by the

CD8+ subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of viral transcription. The invention relates to isolation of clonal CD8+ cells lines that produce the antiviral activity and the development of an assay system for detection of the antiviral activity. The clonal cell lines and the assay system, described herein, may be utilized to purify, characterize and clone the CD8+ suppressor molecule. The CD8+ suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.

CLM What is claimed is:

1. A method for obtaining a preparation containing a CD8+ suppressor molecule, comprising: (a) collecting the conditioned media from cells expressing the CD8+ suppressor molecule; (b) fractionating the media components; and (c) identifying the fractions containing the CD8+ suppressor activity by contacting the fraction with a host cell line transformed with a recombinant DNA expression vector in which a resorter gene is operatively associated with an HIV LTR promoter sequence and measuring the inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with a fraction containing CD8+ suppressor activity.

2. The method of claim 1 in which the CD8+ suppressor molecule is fractionated by chromatography.

3. The method of claim 1 in which the conditioned media is collected from a lymphocyte cell clone that expresses a CD8+ suppressor molecule that inhibits HIV replication.

4. The method of claim 3 in which the conditioned media is collected from the lymphocyte cell clone deposited with the American Type Culture Collection and assigned ATCC Accession No. CRL 11310.

L1 ANSWER 13 OF 16 USPATFULL on STN

1998:119040 Supressor of HIV-1 replication and transcription.

Bolognesi, Dani P., Durham, NC, United States
Chen, Chin-Ho, Durham, NC, United States
Greenberg, Michael, Durham, NC, United States
Weinhold, Kent, Durham, NC, United States
Lacey, Simon F., Durham, NC, United States
Duke University Mdeical Center, Durham, NC, United States (U.S. corporation)

US 5814519 19980929
APPLICATION: US 4885279 19950607 (8)
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a bioactive molecule, herein referred to as the CD8+ suppressor molecule, that is produced by the CD8+ subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of viral transcription. The invention relates to isolation of clonal CD8+ cells lines that produce the antiviral activity and the development of an assay system for detection of the antiviral activity. The clonal cell lines and the assay system, described herein, may be utilized to purify, characterize and clone the CD8+ suppressor molecule. The CD8+ suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.

CLM What is claimed is:

1. A permanently established lymphocyte cell line that expresses the CD8 protein on the cell surface and expresses a CD8+ suppressor molecule that inhibits HIV replication.

2. The lymphocyte cell line of claim 1 wherein said lymphocyte is virally transformed.

3. The lymphocyte cell line of claim 1 wherein said lymphocyte is

virally transformed with Herpes virus.

4. The lymphocyte cell line of claim 3 wherein said cell line is deposited with the American Type Culture collection and assigned accession number CRL 11919.

5. A lymphocyte cell clone that expresses the CD8+ protein on the cell surface and expresses a CD8+ suppressor molecule that inhibits HIV replication.

6. The lymphocyte cell clone of claim 5 deposited with the American Type Culture Collection and assigned accession number CRL 11310.

7. A lymphocyte cell clone that expresses the CD8+ protein on the cell surface and fails to express the CD8+ suppressor molecule, wherein the lymphocyte cell clone is deposited with the American Type Culture Collection and assigned ATCC Accession No. CRL 11309.

L1 ANSWER 14 OF 16 USPATFULL on STN
97:70921 Compounds which inhibit HIV replication.
Wild, Carl T., Durham, NC, United States
Matthews, Thomas J., Durham, NC, United States
Bolognesi, Dani P., Durham, NC, United States
Duke University, Durham, NC, United States (U.S. corporation)
US 5656480 19970812
APPLICATION: US 1995-374666 19950127 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to human immunodeficiency virus (HIV) protein fragments which have antiviral activity, and particularly relates to HIV peptides derived from the HIV transmembrane glycoprotein (gp41) which inhibit HIV-induced cell-cell fusion. This invention further relates to methods for the inhibition of enveloped viral infection, and to methods that modulate biochemical processes which involve coiled coil peptide interactions.
CLM What is claimed is:
1. An isolated peptide selected from the group consisting of: (a) a peptide having the DP-107 amino acid sequence listed in SEQ ID NO:1; (b) a peptide having the DP-125 amino acid sequence listed in SEQ ID NO:3; (c) a peptide having the DP-127 amino acid sequence listed in SEQ ID NO:8; (d) a peptide having the DP-129 amino acid sequence listed in SEQ ID NO:9; (e) a peptide having the DP-130 amino acid sequence listed in SEQ ID NO:10; and (f) a peptide having the DP-137 amino acid sequence listed in SEQ ID NO:12.

2. The peptide of claim 1 wherein the amino terminus of the peptide is acetylated.

3. The peptide of claim 1 wherein the carboxy terminus of the peptide is amidated.

4. An isolated multimer of the peptide of claim 1.

5. The multimer of claim 4 wherein the multimer is a tetramer.

6. The multimer of claim 4 wherein the multimer is a dimer consisting of two peptide monomers.

7. The dimer of claim 5 wherein the monomers of the dimer are covalently bound to one another.

8. A method for inhibiting HIV-induced cell fusion in a culture comprising contacting an human HIV-infected cell with an effective amount of the peptide of claim 1 so that the cell fusion is inhibited.

9. The method of claim 8 wherein the HIV is HIV-1.

10. The method of claim 8 wherein the peptide is present as a multimer.

11. The method of claim 10 wherein the multimer is a dimer having two peptide monomers.

12. A method for inhibiting cell-free HIV transmission in a culture to a human cell not infected with HIV, comprising contacting the cell with a concentration of the peptide of claim 1 so that transmission of the cell-free HIV to the cell not infected with HIV is inhibited.

13. The method of claim 12 wherein the HIV is HIV-1.

14. The method of claim 12 wherein the peptide is present as a multimer.

15. The method of claim 14 wherein the multimer is a dimer having two peptide monomers.

16. The method of claim 15 wherein the monomers are covalently bound to one another.

L1 ANSWER 15 OF 16 USPATFULL on STN
97:38367 Suppressor of HIV replication and transcription.
Bolognesi, Dani P., Durham, NC, United States
Chen, Chin-Ho, Chapel Hill, NC, United States
Greenberg, Michael L., Durham, NC, United States
Weinhold, Kent, Durham, NC, United States
Duke University, Durham, NC, United States (U.S. corporation)
US 5627023 19970506
APPLICATION: US 1993-38387 19930329 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a bioactive molecule, herein referred to as the CD8 suppressor molecule, that is produced by the CD8 subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of vital transcription. The invention relates to isolation of clonal CD8 cells lines that produce the antiviral activity and the development of an assay system for detection of the antiviral activity. The clonal cell lines and the assay system, described herein, may be utilized to purify, characterize and clone the CD8 suppressor molecule. The CD8 suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.
CLM What is claimed is:
1. A method for detecting a CD8 suppressor molecule that has anti-HIV activity, comprising: (a) culturing a host cell line transfected with a recombinant DNA expression vector in which a reporter gene is operatively associated with an HIV LTR promoter sequence; (b) contacting the cell line with a sample comprising enriched CD8 cells or cell cultures of CD8 cells; and (c) measuring the inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with anti-HIV activity.

2. A diagnostic assay for monitoring the clinical progression of HIV infection comprising: (a) collecting successive blood samples from an HIV infected individual; (b) culturing a host cell line transfected with a recombinant DNA expression vector in which a reporter gene is operatively associated with an HIV LTR promoter sequence; (c) contacting the cell line with the samples from the HIV infected individual; (d) measuring the inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with anti-HIV activity; and (e) comparing the inhibition of reporter gene activity in each of the samples wherein a decrease in reporter gene activity in each of the successive samples indicates progression of HIV infection.

3. The method of claim 1 or 2 in which the reporter gene is the chloramphenicol acetyltransferase gene.

4. The method of claim 1 or 2 in which the reporter gene is the firefly luciferase gene.

5. The method of claim 1 or 2 in which the reporter gene is the human growth hormone gene.

L1 ANSWER 16 OF 16 USPATFULL on STN
95:99246 Synthetic peptide inhibitors of HIV transmission.
Bolognesi, Dani P., Durham, NC, United States
Matthews, Thomas J., Durham, NC, United States
Wild, Carl T., Durham, NC, United States
Duke University, Durham, NC, United States (U.S. corporation)
US 5464933 19951107
APPLICATION: US 1993-73028 19930607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to peptides which exhibit potent anti-retroviral activity. The peptides of the invention comprise DP-178 (SEQ ID:1) ptides corresponding to amino acids 638 to 673 of the HIV-1_{LAI} gp41 protein, and fragments, analogs and homologs of DP-178. The invention further relates to the uses of such peptides as inhibitory of human and non-human retroviral, especially HIV, transmission to uninfected cells.
CLM What is claimed is:
1. A peptide having a formula selected from the group consisting of:
X-YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF-Z (SEQ ID: 1)
X-YTNTIYTLLEESQNQQEKNEQELLELDKWASLWNWF-Z (SEQ ID NO:3)
X-YTGIIYNLLEESQNQQEKNEQELLELDKWANLWNWF-Z (SEQ ID NO:4)
X-YTSLIYSLLEKSQIQQEKNEQELLELDKWASLWNWF-Z (SEQ ID NO:5)
X-LEANISKSLEQAQIQQEKNMYELQKLNSWDIFGNWF-Z (SEQ ID NO:6) and
X-LEANISQSLEQAQIQQEKNMYELQKLNSWDVFTNWL-Z (SEQ ID NO:7), in which: amino acid residues are presented by the single-letter code; X comprises an amino group, an acetyl group, a 9-fluorenylmethoxy-carbonyl group, a hydrophobic group, or a macromolecule carrier group; Z comprises a carboxyl group, an amido group, a hydrophobic group, or a macromolecular carrier group.

2. The peptide of claim 1 wherein X is a hydrophobic group.

3. The peptide of claim 2 wherein the hydrophobic group X is carbobenzoxyl, dansyl, or t-butyloxycarbonyl.

4. The peptide of claim 1 wherein Z is a hydrophobic group.

5. The peptide of claim 4 wherein the hydrophobic group Z is t-butyloxycarbonyl.

6. The peptide of claim 1 wherein X is a macromolecular carrier group.

7. The peptide of claim 6 wherein the macromolecular carrier group is a lipid-fatty acid conjugate, a polyethylene glycol, or a carbohydrate moiety.

8. The peptide of claim 1 wherein Z is a macromolecular carrier group.

9. The peptide of claim 8 wherein the macromolecular carrier group Z is a lipid-fatty acid conjugate, a polyethylene glycol, or a carbohydrate moiety.

10. The peptide of claim 1 wherein at least one bond linking adjacent amino acid residues is a non-peptide bond.

11. The peptide of claim 10 wherein the non-peptide bond is an imino, ester, hydrazine, semicarbazide, or azo bond.

12. The peptide of claim 1 wherein at least one amino acid residue is in a D-isomer configuration.

13. The peptide of claim 1 further comprising at least one amino acid substitution wherein a first amino acid residue is substituted for a second, different amino acid residue.

14. The peptide of claim 13 wherein the amino acid substitution is a conserved substitution.

15. The peptide of claim 13 wherein the amino acid substitution is a non-conserved substitution.

16. The peptide homolog of claim 1 wherein the HIV retrovirus is a HIV-1 retrovirus.

17. The peptide homolog of claim 16 wherein the HIV-1 retrovirus is $HIV\text{-}1_{SF2}$.

18. The peptide homolog of claim 16 wherein the retrovirus is $HIV\text{-}1_{RF}$.

19. The peptide homolog of claim 16 wherein the retrovirus is $HIV\text{-}1_{MN}$.

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=> d hi
'HI' IS NOT A VALID FORMAT FOR FILE 'USPATFULL'

The following are valid formats:

The default display format is STD.

ABS -------- AB
ALL -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD,
             RLI, PRAI, DT, FS, REP, REN, EXNAM, LREP, CLMN, ECL,
             DRWN, AB, GOVI, PARN, SUMM, DRWD, DETD, CLM, INCL,
             INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
             EXF, ARTU
ALLG ------- ALL plus PAGE.DRAW
BIB -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD, RLI,
             PRAI, DT, FS, EXNAM, LREP, CLMN, ECL, DRWN, LN.CNT
BIB.EX ----- BIB for original and latest publication
BIBG ------- BIB plus PAGE.DRAW
BROWSE ----- See "HELP BROWSE" or "HELP DISPLAY BROWSE".  BROWSE must
             entered on the same line as DISPLAY, e.g., D BROWSE.
CAS -------- OS, CC, SX, ST, IT
CBIB ------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PRAI, DT, FS
DALL ------- ALL, delimited for post-processing
FP --------- PI, TI, IN, INA, PA, PAA, PAT, PTERM, DCD, AI, RLI,
             PRAI, IC, ICM, ICS, INCL, INCLM, INCLS, NCL,
             NCLM, NCLS, EXF, REP, REN, ARTU, EXNAM, LREP,
             CLMN, DRWN, AB
FP.EX ------ FP for original and latest publication
FPALL ------ PI, TI, IN, INA, PA, PAA, PAT, PETRM, DCD, AI,
             RLI, PRAI, IC, ICM, ICS, INCL, INCLM, INCLS, NCL, NCLM,
             NCLS, EXF, REP, REN,ARTU, EXNAM, LREP, CLMN, DRWN, AB,
             PARN, SUMM, DRWD, DETD, CLM
FPBIB ------ PI, TI, IN, INA, PA, PAA, PAT, PTERM, DCD, AI,
             RLI, PRAI, REP, REN, EXNAM, LREP, CLM, CLMN, DRWN
FHITSTR ---- HIT RN, its text modification, its CA index name, and
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           its structure diagram
G -------- FP plus PAGE.DRAW
 --------- PN and page image numbers
T -------- All fields containing hit terms
TRN ------ HIT RN and its text modification
TSTR ----- HIT RN, its text modification, its CA index name, and
           its structure diagram
BS ------- ABS, indented with text labels
LL ------- ALL, indented with text labels
LLG ------ IALL plus PAGE.DRAW
IB ------- BIB, indented with text labels
IB.EX ---- IBIB for original and latest publication
IBG ------ IBIB plus PAGE.DRAW
AX ------- MAX, indented with text labels
AX.EX ---- IMAX for original and latest publication
D -------- INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
           EXF, ARTU, OS, CC, SX, ST, IT
TD ------- STD, indented with text labels
IC ------- All hit terms plus 20 words on either side
X -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD,
           RLI, PRAI, DT, FS, REP, REN, EXNAM, LREP, CLMN, ECL,
           DRWN, AB, GOVI, PARN, SUMM, DRWD, DETD, CLM, INCL,
           INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
           EXF, ARTU OS, CC, SX, ST, IT
X.EX ----- MAX for original and latest publication
C -------- List of display fields containing hit terms
IB ------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, RLI, PRAI,
           DT, FS, LN.CNT
AN ------- AN, TI, NCL, NCLM, NCLS, IC, ICM, ICS (random display
           without answer number.  SCAN must be entered on the
           same line as DISPLAY, e.g., D SCAN)
D -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, RLI, PRAI,
           DT, FS, LN.CNT, INCL, INCLM, INCLS, NCL, NCLM, NCLS,
           IC, ICM, ICS, EXF (STD is the default)
D.EX ----- STD for original and latest publication
IAL ------ AN, TI, INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC,
           ICM, ICS

TER DISPLAY FORMAT (STD):ti

   ANSWER 1 OF 16  USPATFULL on STN
     Nucleic acids encoding DP-178 and other viral fusion inhibitor peptides
     useful for treating aids


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   FILE 'USPATFULL' ENTERED AT 18:20:33 ON 21 FEB 2004
              E BOLOGNESI DANI P/IN
           16 S E3 OR E4

 d l1,cbib,ab,clm,5,8,12,13,15

   ANSWER 5 OF 16  USPATFULL on STN
03:60103 Suppressor of HIV replication and transcription.
  Bolognesi, Dani P., Durham, NC, United States
  Greenberg, Michael L., Durham, NC, United States
  Lacey, Simon F., Azusa, CA, United States
  Tomaras, Georgia D., Durham, NC, United States
  Weinhold, Kent J., Durham, NC, United States
  Duke University, Durham, NC, United States (U.S. corporation)
  US 6528308 B1 20030304
  APPLICATION: US 2000-527320  20000316 (9)
  DOCUMENT TYPE: Utility; GRANTED.
```

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a bioactive molecule, herein referred to as the CD8+ suppressor molecule, that is produced by the CD8+ subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of viral transcription. The invention relates to isolation of CD8+ cell lines and cell clones that produce that antiviral activity and to the development of assay systems for detection of the antiviral activity. The cell lines, cell clones and assay systems, described herein, may be utilized, e.g., to purify, characterize and clone the CD8+ suppressor molecule. The CD8+ suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.

CLM What is claimed is:

1. A permanently established lymphocyte cell line identified as DU.JR-HVS (ATCC Accession No. PTA-1551).

2. A permanently established lymphocyte cell line identified as DU.HS-HVS (ATCC Accession No. PTA-1552).

L1 ANSWER 8 OF 16 USPATFULL on STN

2002:191473 Suppressor of HIV replication and transcription.

Bolognesi, Dani P., Durham, NC, UNITED STATES
Greenberg, Michael L., Durham, NC, UNITED STATES
Lacey, Simon F., Azusa, CA, UNITED STATES
Tomaras, Georgia D., Durham, NC, UNITED STATES
Weinhold, Kent J., Durham, NC, UNITED STATES
Duke University (U.S. corporation)
US 2002102538 A1 20020801
APPLICATION: US 2002-71349 A1 20020206 (10)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a bioactive molecule, herein referred to as the CD8+ suppressor molecule, that is produced by the CD8+ subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of viral transcription. The invention relates to isolation of CD8+ cell lines and cell clones that produce that antiviral activity and to the development of assay systems for detection of the antiviral activity. The cell lines, cell clones and assay systems, described herein, may be utilized, e.g., to purify, characterize and clone the CD8+ suppressor molecule. The CD8+ suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.

CLM What is claimed is:

1. A method for detecting a CD8+ suppressor molecule that has anti-HIV-1 activity, the method comprising: (a) contacting a host cell with a replication deficient HIV pseudotyped virus comprising a reporter gene operatively associated with an HIV promoter; (b) contacting the host cell with: (i) a sample comprising enriched CD8+ cells; or (ii) a sample comprising a cell culture of CD8+ cells; or (iii) an extract or media component from (i) or (ii); and (c) measuring reporter gene activity, wherein inhibition of reporter gene activity indicates anti-HIV-1 activity.

2. The method of claim 1, wherein the reporter gene is expressed during early proviral gene expression.

3. The method of claim 2, wherein the reporter gene is expressed in place of an early proviral gene.

4. The method of claim 3, wherein the early proviral gene is a nef gene.

5. The method of claim 1, wherein the pseudotyped virus is an env deficient pseudotyped virus.

6. The method of claim 5, wherein the pseudotyped virus is produced by a method comprising co-transfecting DNA for the pseudotyped virus with a vector that encodes a viral envelope protein.

7. The method of claim 6, wherein the viral envelope protein is an HIV Env protein.

8. The method of claim 6, wherein the viral envelope protein is a non-HIV viral envelope protein.

9. The method of claim 1, wherein the reporter gene is a luciferase gene, a chloramphenicol acetyltransferase gene, a growth hormone gene, or a fluorescent protein gene.

10. The method of claim 9, wherein the reporter gene is a luciferase gene.

11. A method for detecting a CD8+ suppressor molecule that has anti-HIV-1 activity, said method comprising: (a) contacting a host cell with an env deficient HIV pseudotyped virus comprising a reporter gene substituted for an HIV nef gene such that said reporter gene is expressed in place of the HIV nef gene; (b) contacting the host cell with: (i) a sample comprising enriched CD8+ cells; or (ii) a sample comprising a cell culture of CD8+ cells; or (iii) an extract or media component from (i) or (ii); and (c) measuring reporter gene activity, wherein inhibition of reporter gene activity indicates anti-HIV-1 activity.

12. The method of claim 11, wherein the reporter gene is a luciferase gene, a chloramphenicol acetyltransferase gene, a growth hormone gene, or a fluorescent protein gene.

13. The method of claim 12, wherein the reporter gene is a luciferase gene.

14. A diagnostic assay for monitoring clinical progression of HIV infection, the diagnostic assay comprising: (a) contacting a host cell with a replication deficient HIV pseudotyped virus comprising a reporter gene operatively associated with an HIV promoter; (b) contacting the host cell with samples from an HIV infected individual, wherein the samples are collected from the individual at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals, wherein an increase in reporter gene activity indicates progression of HIV infection.

15. The method of claim 14, wherein the reporter gene is expressed during early proviral gene expression.

16. The method of claim 15, wherein the reporter gene is expressed in place of an early proviral gene.

17. The method of claim 16, wherein the early proviral gene is a nef gene.

18. The method of claim 16, wherein the pseudotyped virus is an env deficient pseudotyped virus.

19. The method of claim 18, wherein the pseudotyped virus is produced by a method comprising co-transfecting DNA for said pseudotyped virus with a vector that encodes a viral envelope protein.

20. The method of claim 19, wherein the viral envelope protein is an HIV Env protein.

21. The method of claim 19, wherein the viral envelope protein is a

non-HIV viral envelope protein.

22. The method of claim 14, wherein the reporter gene is a chloramphenicol acetyltransferase gene, a luciferase gene, a growth hormone gene, or a fluorescent protein gene.

23. The method of claim 22, wherein the reporter gene is a luciferase gene.

24. A diagnostic assay for monitoring clinical progression of HIV infection, the diagnostic assay comprising: (a) contacting a host cell with an env deficient HIV pseudotyped virus comprising a reporter gene substituted for an HIV nef gene such that said reporter gene is expressed in place of the HIV nef gene; (b) contacting the host cell with samples from an HIV infection individual, wherein the samples are collected from the individual at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals, wherein an increase in reporter gene activity indicates progression of HIV infection.

25. The method of claim 24, wherein the reporter gene is a chloramphenicol acetyltransferase gene, a luciferase gene, a growth hormone gene, or a fluorescent protein gene.

26. The method of claim 25, wherein the reporter gene is a luciferase gene.

27. A method for detecting a compound that suppresses HIV-1 replication, the method comprising: (a) contacting a host cell with a replication deficient HIV pseudotyped virus comprising a reporter gene operatively associated with an HIV promoter; (b) contacting the host cell with the compound at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals, wherein inhibition of reporter gene activity at one or more time intervals indicates that the compound suppresses HIV-1 replication.

28. The method of claim 27, wherein the reporter gene is expressed during early proviral gene expression.

29. The method of claim 28, wherein the reporter gene is expressed in place of an early proviral gene.

30. The method of claim 29, wherein the early proviral gene is a nef gene.

31. The method of claim 27, wherein the pseudotyped virus in an env deficient pseudotyped virus.

32. The method of claim 31, wherein the pseudotyped virus is produced by a method comprising co-transfecting DNA for the pseudotyped virus with a vector that encodes a viral envelope protein.

33. The method of claim 32, wherein the viral envelope protein is an HIV Env protein.

34. The method of claim 32, wherein the viral envelope protein is a non-HIV envelope protein.

35. The method of claim 27, wherein suppression of HIV-1 is at a stage of viral entry.

36. The method of claim 35 further comprising the steps of: (a) contacting a different host cell with the HIV pseudotyped virus; (b) contacting the different host cell with a viral entry inhibitor at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals; wherein the time intervals at which reporter

gene activity is inhibited correspond to time intervals of viral entry.

37. The method of claim 36, wherein the viral entry inhibitor is an anti-fusion peptide.

38. The method of claim 37, wherein the anti-fusion peptide is DP107, DP178, T1249 or T649.

39. The method of claim 36, wherein the viral entry inhibitor is an antibody that disrupts the interaction between a CD4+ cell surface receptor and a viral envelope protein.

40. The method of claim 39, wherein the antibody is a monoclonal antibody that specifically binds to the CD4+ receptor.

41. The method of claim 27, wherein suppression of HIV-1 is at a stage of reverse transcription.

42. The method of claim 41 further comprising the steps of: (a) contacting a different host cell with the HIV pseudotyped virus; (b) contacting the different host cell with a reverse transcription inhibitor at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals; wherein the time intervals at which reporter gene activity is inhibited correspond to time intervals of reverse transcription.

43. The method of claim 42, wherein the reverse transcription inhibitor is a non-nucleoside reverse transcriptase inhibitor.

44. The method of claim 43, wherein the reverse transcriptase inhibitor is nevirapine.

45. The method of claim 27, wherein suppression of HIV-1 is at a stage of early virus gene expression.

46. The method of claim 45 further comprising the steps of: (a) contacting a different host cell with the HIV pseudotyped virus; (b) contacting the different host cell with an inhibitor of early virus gene expression at one or more time intervals; and (c) measuring reporter gene activity at one or more time intervals, wherein the time intervals at which reporter gene activity is inhibited correspond to time intervals of early virus gene expression.

47. The method of claim 46, wherein the inhibitor of early virus gene expression is a Tat inhibitor.

1 ANSWER 12 OF 16 USPATFULL on STN
999:7476 Suppressor of HIV replication and transcription.
Bolognesi, Dani P., Durham, NC, United States
Chen, Chin-Ho, Durham, NC, United States
Greenberg, Michael, Durham, NC, United States
Weinhold, Kent, Durham, NC, United States
Lacey, Simon F., Durham, NC, United States
Duke University Medical Center, Durham, NC, United States (U.S. corporation)
US 5861490 19990119
APPLICATION: US 1995-471430 19950606 (8)
DOCUMENT TYPE: Utility; Granted.
AS INDEXING IS AVAILABLE FOR THIS PATENT.
B The present invention relates to a bioactive molecule, herein referred to as the CD8+ suppressor molecule, that is produced by the CD8+ subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of viral transcription. The invention relates to isolation of clonal CD8+ cells lines that produce the antiviral activity and the development of

an assay system for detection of the antiviral activity. The clonal cell lines and the assay system, described herein, may be utilized to purify, characterize and clone the CD8+ suppressor molecule. The CD8+ suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.

LM What is claimed is:

1. A method for obtaining a preparation containing a CD8+ suppressor molecule, comprising: (a) collecting the conditioned media from cells expressing the CD8+ suppressor molecule; (b) fractionating the media components; and (c) identifying the fractions containing the CD8+ suppressor activity by contacting the fraction with a host cell line transformed with a recombinant DNA expression vector in which a resorter gene is operatively associated with an HIV LTR promoter sequence and measuring the inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with a fraction containing CD8+ suppressor activity.

2. The method of claim 1 in which the CD8+ suppressor molecule is fractionated by chromatography.

3. The method of claim 1 in which the conditioned media is collected from a lymphocyte cell clone that expresses a CD8+ suppressor molecule that inhibits HIV replication.

4. The method of claim 3 in which the conditioned media is collected from the lymphocyte cell clone deposited with the American Type Culture Collection and assigned ATCC Accession No. CRL 11310.

1 ANSWER 13 OF 16 USPATFULL on STN

998:119040 Supressor of HIV-1 replication and transcription.

Bolognesi, Dani P., Durham, NC, United States
Chen, Chin-Ho, Durham, NC, United States
Greenberg, Michael, Durham, NC, United States
Weinhold, Kent, Durham, NC, United States
Lacey, Simon F., Durham, NC, United States
Duke University Mdeical Center, Durham, NC, United States (U.S. corporation)

US 5814519 19980929
APPLICATION: US 4885279 19950607 (8)
DOCUMENT TYPE: Utility; Granted.

AS INDEXING IS AVAILABLE FOR THIS PATENT.

B The present invention relates to a bioactive molecule, herein referred to as the CD8+ suppressor molecule, that is produced by the CD8+ subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of viral transcription. The invention relates to isolation of clonal CD8+ cells lines that produce the antiviral activity and the development of an assay system for detection of the antiviral activity. The clonal cell lines and the assay system, described herein, may be utilized to purify, characterize and clone the CD8+ suppressor molecule. The CD8+ suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.

LM What is claimed is:

1. A permanently established lymphocyte cell line that expresses the CD8 protein on the cell surface and expresses a CD8+ suppressor molecule that inhibits HIV replication.

2. The lymphocyte cell line of claim 1 wherein said lymphocyte is virally transformed.

3. The lymphocyte cell line of claim 1 wherein said lymphocyte is virally transformed with Herpes virus.

4. The lymphocyte cell line of claim 3 wherein said cell line is deposited with the American Type Culture collection and assigned

accession number CRL 11919.

5. A lymphocyte cell clone that expresses the CD8+ protein on the cell surface and expresses a CD8+ suppressor molecule that inhibits HIV replication.

6. The lymphocyte cell clone of claim 5 deposited with the American Type Culture Collection and assigned accession number CRL 11310.

7. A lymphocyte cell clone that expresses the CD8+ protein on the cell surface and fails to express the CD8+ suppressor molecule, wherein the lymphocyte cell clone is deposited with the American Type Culture Collection and assigned ATCC Accession No. CRL 11309.

L1 ANSWER 15 OF 16 USPATFULL on STN
97:38367 Suppressor of HIV replication and transcription.
Bolognesi, Dani P., Durham, NC, United States
Chen, Chin-Ho, Chapel Hill, NC, United States
Greenberg, Michael L., Durham, NC, United States
Weinhold, Kent, Durham, NC, United States
Duke University, Durham, NC, United States (U.S. corporation)
US 5627023 19970506
APPLICATION: US 1993-38387 19930329 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a bioactive molecule, herein referred to as the CD8 suppressor molecule, that is produced by the CD8 subset of human T-lymphocytes and suppresses type-1 human immunodeficiency virus (HIV-1) replication through inhibition of vital transcription. The invention relates to isolation of clonal CD8 cells lines that produce the antiviral activity and the development of an assay system for detection of the antiviral activity. The clonal cell lines and the assay system, described herein, may be utilized to purify, characterize and clone the CD8 suppressor molecule. The CD8 suppressor molecule may have therapeutic applications for treatment of diseases associated with HIV-1 infection.
CLM What is claimed is:
1. A method for detecting a CD8 suppressor molecule that has anti-HIV activity, comprising: (a) culturing a host cell line transfected with a recombinant DNA expression vector in which a reporter gene is operatively associated with an HIV LTR promoter sequence; (b) contacting the cell line with a sample comprising enriched CD8 cells or cell cultures of CD8 cells; and (c) measuring the inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with anti-HIV activity.

2. A diagnostic assay for monitoring the clinical progression of HIV infection comprising: (a) collecting successive blood samples from an HIV infected individual; (b) culturing a host cell line transfected with a recombinant DNA expression vector in which a reporter gene is operatively associated with an HIV LTR promoter sequence; (c) contacting the cell line with the samples from the HIV infected individual; (d) measuring the inhibition of reporter gene activity, wherein inhibition of reporter gene activity correlates with anti-HIV activity; and (e) comparing the inhibition of reporter gene activity in each of the samples wherein a decrease in reporter gene activity in each of the successive samples indicates progression of HIV infection.

3. The method of claim 1 or 2 in which the reporter gene is the chloramphenicol acetyltransferase gene.

4. The method of claim 1 or 2 in which the reporter gene is the firefly luciferase gene.

5. The method of claim 1 or 2 in which the reporter gene is the human

growth hormone gene.

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=> s l4 not (l1 or l2)
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E7            2     WEINHOLD OTTO/IN
E8            1     WEINHOLD ROBERT B/IN
E9            1     WEINHOLD ROLF/IN
E10           5     WEINHOLD STEPHEN/IN
E11           1     WEINHOLD THOMAS/IN
E12           2     WEINHOLD WOLFGANG/IN

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              2 "WEINHOLD KENT J"/IN
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L7            0 L6 NOT (L1 OR L2)
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     (FILE 'HOME' ENTERED AT 18:20:00 ON 21 FEB 2004)

     FILE 'USPATFULL' ENTERED AT 18:20:33 ON 21 FEB 2004
                E BOLOGNESI DANI P/IN
L1             16 S E3 OR E4
                E GREENBERG MICHAEL L/IN
L2              3 S E3
L3              0 S L2 NOT L1
                E LACEY SIMON F/IN
L4              6 S E3
L5              0 S L4 NOT (L1 OR L2)
                E WEINHOLD KENT J/IN
L6              7 S E2 OR E3
L7              0 S L6 NOT (L1 OR L2)

=> s (HIV or human immunodeficiency virus)
         29336 HIV
        372979 HUMAN
         16965 IMMUNODEFICIENCY
         70100 VIRUS
         12163 HUMAN IMMUNODEFICIENCY VIRUS
                 (HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L8       30918 (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> s l8 and (CD8?)
          8280 CD8?
L9        4673 L8 AND (CD8?)

=> s l9 and (CD8? T lymphocyte? or CD8? T cell?)
          8280 CD8?
        918824 T
         39046 LYMPHOCYTE?
           340 CD8? T LYMPHOCYTE?
                 (CD8?(W)T(W)LYMPHOCYTE?)
          8280 CD8?
        918824 T
        738345 CELL?
          1619 CD8? T CELL?
                 (CD8?(W)T(W)CELL?)
L10       1125 L9 AND (CD8? T LYMPHOCYTE? OR CD8? T CELL?)

=> s l10 and (inhibit? or suppress? or impair?)
        448282 INHIBIT?
        263253 SUPPRESS?
        149400 IMPAIR?
L11       1076 L10 AND (INHIBIT? OR SUPPRESS? OR IMPAIR?)

=> s l11 and (soluble inhibitor? or soluble factor?)
        337470 SOLUBLE
        175234 INHIBITOR?
           214 SOLUBLE INHIBITOR?
                 (SOLUBLE(W)INHIBITOR?)
        337470 SOLUBLE
        775385 FACTOR?
          1555 SOLUBLE FACTOR?
                 (SOLUBLE(W)FACTOR?)
L12         69 L11 AND (SOLUBLE INHIBITOR? OR SOLUBLE FACTOR?)

=> s l12 and (inhibit?/clm or suppress?/clm or impair?/clm)
        100299 INHIBIT?/CLM
         25788 SUPPRESS?/CLM
          3837 IMPAIR?/CLM
L13         29 L12 AND (INHIBIT?/CLM OR SUPPRESS?/CLM OR IMPAIR?/CLM)
```

=> s l13 not l1
L14 27 L13 NOT L1

=> d l14,cbib,ab,clm,1-27

L14 ANSWER 1 OF 27 USPATFULL on STN
2004:21474 Human monoclonal antibodies to CTLA-4.
Hanson, Douglas Charles, Niantic, CT, United States
Neveu, Mark Joseph, Mystic, CT, United States
Mueller, Eileen Elliott, Old Lyme, CT, United States
Hanke, Jeffrey Herbert, Reading, MA, United States
Gilman, Steven Christopher, Old Lyme, CT, United States
Davis, C. Geoffrey, Burlingame, CA, United States
Corvalan, Jose Ramon, Foster City, CA, United States
Abgenix, Inc., Fremont, CA, United States (U.S. corporation) Pfizer Inc., Fremont, CA, United States (U.S. corporation)
US 6682736 B1 20040127
APPLICATION: US 1999-472087 19991223 (9)
PRIORITY: US 1998-113647P 19981223 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB In accordance with the present invention, there are provided fully human monoclonal antibodies against human cytotoxic T-lymphocyte antigen 4 (CTLA-4). Nucelotide sequences encoding and amino acid sequences comprising heavy and light chain immunoglobulin molecules, particularly contiguous heavy and light chain sequences spanning the complementarity determining regions (CDRs), specifically from within FR1 and/or CDR1 through CDR3 and/or within FR4, are provided. Further provided are antibodies having similar binding properties and antibodies (or other antagonists) having similar functionality as antibodies disclosed herein.
CLM What is claimed is:
1. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, comprising a heavy chain variable region amino acid sequence that comprises a contiguous amino acid sequence from within an FR1 sequence through an FR3 sequence that utilizes a human V_{H3-33} family gene and that comprises at least one of the amino acid substitutions in any one of SEQ ID NO: 74-79 and 81-84 as compared to SEQ ID NO: 72.

2. The monoclonal antibody or antigen-binding portion thereof of claim 1, wherein the heavy chain variable region amino acid sequence comprises amino acid residues 1-89 of SEQ ID NO: 9.

3. The monoclonal antibody or antigen-binding portion thereof of claim 2, further comprising a light chain variable region amino acid sequence that comprises the variable region of SEQ ID NO: 22.

4. An isolated human monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, wherein the antibody or antigen-binding portion thereof has a binding affinity that is about 10^{-9} M, or greater affinity; possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1; **inhibits** binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM; and **inhibits** binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM.

5. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof competes for binding with CTLA-4 with an antibody comprising a heavy chain amino acid sequence comprising amino acid residues 1-89 of SEQ ID NO: 9 and a light chain amino acid sequence comprising SEQ ID NO: 22.

6. An isolated human monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, wherein the antibody or antigen-binding portion thereof has a binding affinity that is about

10^{-9} M, or greater affinity; possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1; **inhibits** binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM; and **inhibits** binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM; and cross competes for binding with CTLA-4 with an antibody comprising a heavy chain amino acid sequence comprising amino acid residues 1-89 of SEQ ID NO: 9 and a light chain amino acid sequence comprising SEQ ID NO: 22.

7. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof is not cross reactive with CTLA-4 from lower mammalian species.

8. The monoclonal antibody or antigen-binding portion thereof of claim 7, wherein the lower mammalian species comprises mouse, rat, and rabbit.

9. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof is cross reactive with CTLA-4 from primates.

10. The monoclonal antibody or antigen-binding portion thereof of claim 9, wherein the primates comprise cynomolgous and rhesus monkeys.

11. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the selectivity is about 500:1 or greater.

12. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the binding affinity of the monoclonal antibody or antigen-binding portion thereof is about 10^{-10} M, or greater affinity.

13. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof **inhibits** binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 0.38 nM.

14. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof **inhibits** binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 0.50 nM.

15. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof enhances IL-2 production in a T cell blast/Raji assay by about 500 pg/ml or greater.

16. The monoclonal antibody or antigen-binding portion thereof of claim 15, wherein the monoclonal antibody or antigen-binding portion thereof enhances IL-2 production in a T cell blast/Raji assay by about 3846 pg/ml or greater.

17. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof enhances IFN-γ production in a T cell blast/Raji assay by about 500 pg/ml or greater.

18. The monoclonal antibody or antigen-binding portion thereof of claim 17, wherein the monoclonal antibody or antigen-binding portion thereof enhances IFN-γ production in a T cell blast/Raji assay by about 1233 pg/ml or greater.

19. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof induces IL-2 production in a hPBMC or whole blood superantigen assay by about 500 pg/ml or greater.

20. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof induces IL-2 production in a hPBMC or whole blood superantigen assay by about 1500 pg/ml or greater.

21. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof induces IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 30% relative to control.

22. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof induces IL-2 production in a hPBMC or whole blood superantigen assay by greater than about 50% relative to control.

23. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences that utilize a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, the CDR1 sequence being independently selected from amino acid residues 17-26 of SEQ ID NO: 72, 73, 74, 75, 76, 9, 81 or 83, amino acid residues 13-22 of SEQ ID NO: 77 or 78, amino acid residues 18-27 of SEQ ID NO: 79, amino acid residues 16-25 of SEQ ID NO: 82, or amino acid residues 15-24 of SEQ ID NO: 84; the CDR2 sequence being independently selected from amino acid residues 41-55 of SEQ ID NO: 72, 73, 74, 75, 76, 9, 81 or 83, amino acid residues 37-51 of SEQ ID NO: 77 or 78, amino acid residues 42-56 of SEQ ID NO: 79, amino acid residues 40-54 of SEQ ID NO: 82, or amino acid residues 39-53 of SEQ ID NO: 84; and the CDR3 sequence being independently selected from amino acid residues 90-100 of SEQ ID NO: 73 or 81, amino acid residues 90-98 of SEQ ID NO: 74 or 76, amino acid residues 90-99 of SEQ ID NO: 75, amino acid residues 86-94 of SEQ ID NO: 77 or 78, amino acid residues 91-99 of SEQ ID NO: 79, amino acid residues 89-99 of SEQ ID NO: 82, amino acid residues 90-104 of SEQ ID NO: 83, or amino acid residues 88-99 of SEQ ID NO: 84.

24. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences that utilize a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, which antibody or antigen-binding portion thereof has the following properties: a binding affinity for CTLA-4 that is about 10^{-9} M, or greater affinity; **inhibits** binding between CTLA-4 and B7-1 with an IC_{50} of about 100 nM or lower; **inhibits** binding between CTLA-4 and B7-2 with an IC_{50} of about 100 nM or lower; and enhances cytokine production in a human T cell blast/Raji assay by 500 pg/ml or greater.

25. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, comprising a heavy chain amino acid sequence comprising FR1, PR2, and FR3 sequences operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, the CDR1 sequence being independently selected from amino acid residues 17-26 of SEQ ID NO: 72, 73, 74, 75, 76, 9, 81 or 83, amino acid residues 13-22 of SEQ ID NO: 77 or 78, amino acid residues 18-27 of SEQ ID NO: 79, amino acid residues 16-25 of SEQ ID NO: 82, amino acid residues 15-24 of SEQ ID NO: 84, or amino acid residues 24-31 of SEQ ID NO: 86; the CDR2 sequence being independently selected from amino acid residues 41-55 of SEQ ID NO: 72, 73, 74, 75, 76, 9, 81 or 83, amino acid residues 37-51 of SEQ ID NO: 77 or 78, amino acid residues 42-56 of SEQ ID NO: 79, amino acid residues 40-54 of SEQ ID NO: 82, amino acid residues 39-53 of SEQ ID NO: 84, or amino acid residues 44-61 of SEQ ID NO: 86; and the CDR3 sequence being independently selected from amino acid residues 90-100 of SEQ ID NO: 73 or 81, amino acid residues 90-98 of SEQ ID NO: 74 or 76, amino acid

residues 90-99 of SEQ ID NO: 75, amino acid residues 86-94 of SEQ ID NO: 77 or 78, amino acid residues 91-99 of SEQ ID NO: 79, amino acid residues 89-99 of SEQ ID NO: 82, amino acid residues 90-104 of SEQ ID NO: 83, amino acid residues 88-99 of SEQ ID NO: 84, or amino acid residues 94-102 of SEQ ID NO: 86, which antibody or antigen-binding portion thereof has the following properties: a binding affinity for CTLA-4 that is about 10^{-9} M, or greater affinity; **inhibits** binding between CTLA-4 and B7-1 with an IC_{50} of about 100 nM or lower; **inhibits** binding between CTLA-4 and B7-2 with an IC_{50} of about 100 nM or lower; and enhances cytokine production in a human T cell blast/Raji assay by 500 pg/ml or greater.

26. A monoclonal antibody or antigen-binding portion thereof that specifically binds CTLA-4, having a light chain CDR1 sequence, CDR2 sequence and CDR3 sequence, comprising amino acid residues 17-27, 43-49 and 82-90 of SEQ ID NO: 96.

27. The monoclonal antibody or antigen-binding portion thereof of claim 26 comprising the light chain variable region amino acid sequence in SEQ ID NO: 22.

28. The monoclonal antibody or antigen-binding portion thereof of claim 26 comprising a light chain amino acid sequence comprising SEQ ID NO: 22.

29. The monoclonal antibody or antigen-binding portion thereof according to claim 26, comprising amino acid residues 17-90 of SEQ ID NO: 96.

30. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, comprising a heavy chain amino acid sequence comprising amino acid residues 1-89 of SEQ ID NO: 9 and a light chain amino acid sequence comprising SEQ ID NO: 22.

31. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, having a heavy chain CDR1 sequence, CDR2 sequence and CDR3 sequence, comprising amino acid residues 17-26, 41-55 and 90-105 of SEQ ID NO: 80.

32. The monoclonal antibody or antigen-binding portion thereof according to claim 31, further comprising a light chain CDR1 sequence, CDR2 sequence and CDR3 sequence, comprising amino acid residues 17-27, 43-49 and 82-90 of SEQ ID NO: 96.

33. The monoclonal antibody or antigen-binding portion thereof according to claim 32, comprising amino acid residues 17-105 of SEQ ID NO: 80 and amino acid residues 17-90 of SEQ ID NO: 96.

34. The monoclonal antibody or antigen-binding portion thereof of claim 31 comprising a heavy chain variable region amino acid sequence that comprises the variable region amino acid sequence in SEQ ID NO: 70.

35. The monoclonal antibody or antigen-binding portion thereof of claim 34, further comprising a light chain variable region amino acid sequence that comprises the variable region amino acid sequence in SEQ ID NO: 71.

36. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4 comprising a light chain amino acid sequence comprising SEQ ID NO: 71.

37. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4 comprising a heavy chain amino acid sequence comprising SEQ ID NO: 70.

38. A monoclonal antibody that specifically binds to CTLA-4 comprising a heavy chain amino acid sequence comprising SEQ ID NO: 70 and a light chain amino acid sequence comprising SEQ ID NO: 71.

39. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4 comprising a heavy chain variable region amino acid sequence that utilizes a human V_{H3-33} family gene.

40. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the antibody or antigen-binding portion thereof binds to a conformational epitope on human CTLA-4.

41. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the antibody or antigen-binding portion thereof binds to human, cynomologous or marmoset CTLA-4 with about the same on-rate and off-rate.

42. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the antibody or antigen-binding portion thereof **inhibits** human tumor growth.

43. The monoclonal antibody or antigen-binding portion thereof of claim 6, wherein the monoclonal antibody or antigen-binding portion thereof binds to the same epitope on CTLA-4 as an antibody comprising a heavy chain amino acid sequence comprising amino acid residues 1-89 of SEQ ID NO: 9 and a light chain amino acid sequence comprising SEQ ID NO: 22.

44. The monoclonal antibody or antigen-binding portion thereof of claim 4, wherein the monoclonal antibody or antigen-binding portion thereof competes for binding with CTLA-4 with an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO: 70 and a light chain amino acid sequence comprising SEQ ID NO: 71.

45. An isolated human monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, wherein the monoclonal antibody or antigen-binding portion thereof has a binding affinity that is about 10^{-9} M, or greater affinity; possesses a selectivity for CTLA-4 over CD28, B7-2, CD44, and hIgG1 of greater than about 100:1; **inhibits** binding between CTLA-4 and B7-2 with an IC_{50} of lower than about 100 nM; and **inhibits** binding between CTLA-4 and B7-1 with an IC_{50} of lower than about 100 nM; and cross-competes for binding with CTLA-4 with an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO: 70 and a light chain amino acid sequence comprising SEQ ID NO: 71.

46. The monoclonal antibody or antigen-binding portion thereof of claim 45, wherein the monoclonal antibody or antigen-binding portion thereof binds to the same epitope on CTLA-4 as an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO: 70 and a light chain amino acid sequence comprising SEQ ID NO: 71.

47. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, comprising a heavy chain amino acid sequence comprising human FR1, FR2, and FR3 sequences that utilize a human V_H 3-33 gene family operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, the CDR1 sequence being independently selected from amino acid residues 17-26 of SEQ ID NO: 72, 73, 74, 75, 76, 80, 81 or 83, amino acid residues 13-22 of SEQ ID NO: 77 or 78, amino acid residues 18-27 of SEQ ID NO: 79, amino acid residues 16-25 of SEQ ID NO: 82, or amino acid residues 15-24 of SEQ ID NO; 84; the CDR2 sequence being independently selected from amino acid residues 41-55 of SEQ ID NO: 72, 73, 74, 75, 76, 80, 81 or 83, amino acid residues 37-51 of SEQ. ID NO: 77 or 78, amino acid residues 42-56 of SEQ ID NO: 79, amino acid residues 40-54 of SEQ ID NO: 82, or amino acid residues 39-53 of SEQ ID NO: 84; and the CDR3 sequence being independently selected from amino acid residues 90-100 of SEQ ID NO: 73 or 81, amino acid residues 90-98 of SEQ ID NO: 74 or 76, amino acid residues 90-99 of SEQ ID NO: 75, amino acid residues 86-94 of SEQ ID NO: 77 or 78, amino acid

residues 91-99 of SEQ ID NO: 79, amino acid residues 90-105 of SEQ ID NO: 80, amino acid residues 89-99 of SEQ ID NO: 82, amino acid residues 90-104 of SEQ ID NO: 83, or amino acid residues 88-99 of SEQ ID NO: 84.

48. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4, comprising a heavy chain amino acid sequence comprising FR1, FR2, and FR3 sequences operably linked in frame with a CDR1, a CDR2, and a CDR3 sequence, the CDR1 sequence being independently selected from amino acid residues 17-26 of SEQ ID NO: 72, 73, 74, 75, 76, 80, 81 or 83, amino acid residues 13-22 of SEQ ID NO: 77 or 78, amino acid residues 18-27 of SEQ ID NO: 79, amino acid residues 16-25 of SEQ ID NO: 82, amino acid residues 15-24 of SEQ ID NO: 84, or amino acid residues 24-31 of SEQ ID NO: 86; the CDR2 sequence being independently selected from amino acid residues 41-55 of SEQ ID NO: 72, 73, 74, 75, 76, 80, 81 or 83, amino acid residues 37-51 of SEQ ID NO: 77 or 78, amino acid residues 42-56 of SEQ ID NO: 79, amino acid residues 40-54 of SEQ ID NO: 82, amino acid residues 39-53 of SEQ ID NO: 84, or amino acid residues 44-61 of SEQ ID NO: 86; and the CDR3 sequence being independently selected from amino acid residues 90-100 of SEQ ID NO: 73 or 81, amino acid residues 90-98 of SEQ ID NO: 74 or 76, amino acid residues 90-99 of SEQ ID NO: 75, amino acid residues 86-94 of SEQ ID NO: 77 or 78, amino acid residues 91-99 of SEQ ID NO: 79, amino acid residues 90-105 of SEQ ID NO: 80, amino acid residues 89-99 of SEQ ID NO: 82, amino acid residues 90-104 of SEQ ID NO: 83, amino acid residues 88-99 of SEQ ID NO: 84, or amino acid residues 94-102 of SEQ ID NO: 86, which antibody or antigen-binding portion thereof has the following properties: a binding affinity for CTLA-4 that is about 10^{-9} M, or greater affinity; **inhibits** binding between CTLA-4 and B7-1 with an IC_{50} of about 100 nM or lower; **inhibits** binding between CTLA-4 and B7-2 with an IC_{50} of about 100 nM or lower; and enhances cytokine production in a human T cell blast/Raji assay by 500 pg/ml or greater.

49. A monoclonal antibody or antigen-binding portion thereof that specifically binds to CTLA-4 comprising a light chain variable region amino acid sequence that comprises the variable region amino acid sequence in SEQ ID NO: 71.

50. The monoclonal antibody or antigen-binding portion thereof of claim 1 that is a monoclonal antibody.

51. The monoclonal antibody or antigen-binding portion thereof of claim 2 that is a monoclonal antibody.

52. The monoclonal antibody or antigen-binding portion thereof of claim 3 that is a monoclonal antibody.

53. The monoclonal antibody or antigen-binding portion thereof of claim 4 that is a monoclonal antibody.

54. The monoclonal antibody or antigen-binding portion thereof of claim 5 that is a monoclonal antibody.

55. The monoclonal antibody or antigen-binding portion thereof of claim 6 that is a monoclonal antibody.

56. The monoclonal antibody or antigen-binding portion thereof of claim 7 that is a monoclonal antibody.

57. The monoclonal antibody or antigen-binding portion thereof of claim 8 that is a monoclonal antibody.

58. The monoclonal antibody or antigen-binding portion thereof of claim 9 that is a monoclonal antibody.

59. The monoclonal antibody or antigen-binding portion thereof of claim

10 that is a monoclonal antibody.

60. The monoclonal antibody or antigen-binding portion thereof of claim
11 that is a monoclonal antibody.

61. The monoclonal antibody or antigen-binding portion thereof of claim
12 that is a monoclonal antibody.

62. The monoclonal antibody or antigen-binding portion thereof of claim
13 that is a monoclonal antibody.

63. The monoclonal antibody or antigen-binding portion thereof of claim
14 that is a monoclonal antibody.

64. The monoclonal antibody or antigen-binding portion thereof of claim
15 that is a monoclonal antibody.

65. The monoclonal antibody or antigen-binding portion thereof of claim
16 that is a monoclonal antibody.

66. The monoclonal antibody or antigen-binding portion thereof of claim
17 that is a monoclonal antibody.

67. The monoclonal antibody or antigen-binding portion thereof of claim
18 that is a monoclonal antibody.

68. The monoclonal antibody or antigen-binding portion thereof of claim
19 that is a monoclonal antibody.

69. The monoclonal antibody or antigen-binding portion thereof of claim
20 that is a monoclonal antibody.

70. The monoclonal antibody or antigen-binding portion thereof of claim
21 that is a monoclonal antibody.

71. The monoclonal antibody or antigen-binding portion thereof of claim
22 that is a monoclonal antibody.

72. The monoclonal antibody or antigen-binding portion thereof of claim
23 that is a monoclonal antibody.

73. The monoclonal antibody or antigen-binding portion thereof of claim
24 that is a monoclonal antibody.

74. The monoclonal antibody or antigen-binding portion thereof of claim
25 that is a monoclonal antibody.

75. The monoclonal antibody or antigen-binding portion thereof of claim
26 that is a monoclonal antibody.

76. The monoclonal antibody or antigen-binding portion thereof of claim
27 that is a monoclonal antibody.

77. The monoclonal antibody or antigen-binding portion thereof of claim
28 that is a monoclonal antibody.

78. The monoclonal antibody or antigen-binding portion thereof of claim
29 that is a monoclonal antibody.

79. The monoclonal antibody or antigen-binding portion thereof of claim
30 that is a monoclonal antibody.

80. The monoclonal antibody or antigen-binding portion thereof of claim
31 that is a monoclonal antibody.

81. The monoclonal antibody or antigen-binding portion thereof of claim

32 that is a monoclonal antibody.

82. The monoclonal antibody or antigen-binding portion thereof of claim 33 that is a monoclonal antibody.

83. The monoclonal antibody or antigen-binding portion thereof of claim 34 that is a monoclonal antibody.

84. The monoclonal antibody or antigen-binding portion thereof of claim 35 that is a monoclonal antibody.

85. The monoclonal antibody or antigen-binding portion thereof of claim 36 that is a monoclonal antibody.

86. The monoclonal antibody or antigen-binding portion thereof of claim 37 that is a monoclonal antibody.

87. The monoclonal antibody or antigen-binding portion thereof of claim 38 that is a monoclonal antibody.

88. The monoclonal antibody or antigen-binding portion thereof of claim 39 that is a monoclonal antibody.

89. The monoclonal antibody or antigen-binding portion thereof of claim 40 that is a monoclonal antibody.

90. The monoclonal antibody or antigen-binding portion thereof of claim 41 that is a monoclonal antibody.

91. The monoclonal antibody or antigen-binding portion thereof of claim 42 that is a monoclonal antibody.

92. The monoclonal antibody or antigen-binding portion thereof of claim 43 that is a monoclonal antibody.

93. The monoclonal antibody or antigen-binding portion thereof of claim 44 that is a monoclonal antibody.

94. The monoclonal antibody or antigen-binding portion thereof of claim 45 that is a monoclonal antibody.

95. The monoclonal antibody or antigen-binding portion thereof of claim 46 that is a monoclonal antibody.

96. The monoclonal antibody or antigen-binding portion thereof of claim 47 that is a monoclonal antibody.

97. The monoclonal antibody or antigen-binding portion thereof of claim 48 that is a monoclonal antibody.

98. The monoclonal antibody or antigen-binding portion thereof of claim 49 is a monoclonal antibody.

99. The monoclonal antibody of claim 38 that has a heavy chain amino acid sequence of SEQ ID NO: 70 and a light chain amino acid sequence of SEQ ID NO: 71.

100. A composition comprising the monoclonal antibody or antigen-binding portion of claim 4 and a pharmaceutically acceptable carrier.

101. A composition comprising the monoclonal antibody of claim 38 and a pharmaceutically acceptable carrier.

102. A monoclonal antibody or antigen binding portion thereof that specifically binds to CTLA-4, wherein the heavy chain variable region and the light chain variable region are produced by hybridoma cell line

11.2.1.4 deposited under ATCC Accession No. PTA-5169.

103. The monoclonal antibody or antigen-binding portion thereof of claim 102 that is a monoclonal antibody.

104. A monoclonal antibody that specifically binds to CTLA-4 and is produced by hybridoma cell line 11.2.1.4 deposited under ATCC Accession No. PTA-5169.

L14 ANSWER 2 OF 27 USPATFULL on STN
2004:13417 Methods of enhancing immune induction involving MDA-7.
Chada, Sunil, Missouri City, TX, UNITED STATES
Pataer, Abujiang, Houston, TX, UNITED STATES
Mhashilkar, Abner, Houston, TX, UNITED STATES
Ramesh, Rajagopal, Sugarland, TX, UNITED STATES
Roth, Jack, Houston, TX, UNITED STATES
Swisher, Steve, Fresno, TX, UNITED STATES
Board of Regent, The University of Texas System (U.S. corporation) Introgen Therapeutics, Inc. (U.S. corporation)
US 2004009939 A1 20040115
APPLICATION: US 2003-378590 A1 20030303 (10)
PRIORITY: US 2002-404932P 20020821 (60)
US 2002-370335P 20020405 (60)
US 2002-361755P 20020305 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to compositions and methods for the enhancing or inducing an immune response against an immunogenic molecule by indirectly activating PKR. More specifically, immunotherapy is improved by co-administering a MDA-7 polypeptide with an immunogenic molecule against which an immune response is desired. Such immunotherapies include cancer vaccines, and compositions thereof are described.
CLM What is claimed is:
1. An immunogenic composition comprising: (a) an immunogenic molecule or a nucleic acid encoding an immunogenic molecule; and (b) a recombinant MDA-7 polypeptide or an isolated nucleic acid expressing the MDA-7 polypeptide.

2. The composition of claim 1, wherein the composition is in a pharmaceutically acceptable diluent.

3. The composition of claim 1, wherein the immunogenic molecule is an antigen.

4. The composition of claim 3, wherein the antigen is a tumor antigen.

5. The composition of claim 4, wherein the tumor antigen is PSA, CEA, MAGE1, MAGE3, gp100, AFP, her2, tert, muc1, NY-ESO, bcr-abl, trp1, trp2, MART, BAGE, GAGE, or PMSA.

6. The composition of claim 3, wherein the antigen is a microbial, viral or fungal antigen.

7. The composition of claim 1, wherein the immunogenic molecule is at least one polypeptide.

8. The composition of claim 1, wherein the immunogenic molecule is a T-cell activation molecule.

9. The composition of claim 1, wherein the MDA-7 polypeptide comprises amino acids from 49 to 206 of SEQ ID NO:2.

10. The composition of claim 14, wherein the MDA-7 polypeptide comprises the sequence of SEQ ID NO:2.

11. The composition of claim 1 comprising a recombinant MDA-7 polypeptide.

12. The composition of claim 1, wherein the nucleic acid is an expression vector.

13. The composition of claim 12, wherein the expression vector is a viral vector.

14. The composition of claim 13, wherein the viral vector is an adenoviral vector, a retroviral vector, a vaccinia viral vector, an adeno-associated viral vector, a polyoma viral vector, an alphaviral vector, a rhabdoviral vector or a herpes viral vector.

15. The composition of claim 1, wherein the MDA-7 polypeptide comprises a secretory signal.

16. The composition of claim 1, wherein the composition further comprises a colloidal carrier.

17. The immunogenic composition of claim 1, further comprising a cytokine or an isolated nucleic acid encoding the cytokine.

18. A method of promoting an immune response in a patient comprising administering to the patient an effective amount of a MDA-7 polypeptide or a nucleic acid encoding the MDA-7 polypeptide, wherein the MDA-7 polypeptide promotes the immune response in the patient.

19. The method of claim 18, further comprising providing to a patient an immunogenic molecule or a nucleic acid encoding the immunogenic molecule.

20. The method of claim 19, wherein the immune response is against the immunogenic molecule.

21. The method of claim 19, wherein the immunogenic molecule is an antigen.

22. The method of claim 21, wherein the antigen is a tumor antigen.

23. The method of claim 22, wherein the tumor antigen is PSA, CEA, MAGE1, MAGE3, gp100, AFP, her2, tert, muc1, NY-ESO, bcr-ab1, trp1, trp2, MART, BAGE, GAGE, or PMSA.

24. The method of claim 22, wherein i) the immunogenic molecule and ii) the MDA-7 polypeptide or the nucleic acid encoding the MDA-7 are administered before chemotherapy, radiotherapy or surgery.

25. The method of claim 22, wherein the immunogenic molecule and the MDA-7 polypeptide are administered during chemotherapy, radiotherapy or surgery.

26. The method of claim 22, wherein the immunogenic molecule and the MDA-7 are administered to the patient after chemotherapy, radiotherapy or surgery.

27. The method of claim 21, wherein the antigen is a microbial, viral, or fungal antigen.

28. The method of claim 19, wherein the immunogenic molecule comprises at least one polypeptide.

29. The method of claim 18, further comprising identifying a patient in need of promoting an immune response.

30. The method of claim 18, wherein the MDA-7 is provided to the patient by administering a vector comprising an isolated nucleic acid sequence encoding the MDA-7 polypeptide.

31. The method of claim 30, wherein the vector is an expression vector.

32. The method of claim 31, wherein the expression vector is a viral vector.

33. The method of claim 32, wherein the viral vector is an adenoviral vector, a retroviral vector, a vaccinia viral vector, an adeno-associated viral vector, a polyoma viral vector, an alphaviral vector, a rhabdoviral vector or a herpes viral vector.

34. The method of claim 30, wherein the vector further comprises the nucleic acid sequence encoding the immunogenic molecule.

35. The method of claim 18, further comprising detecting the immune response.

36. The method of claim 35, wherein the immune response comprises increasing activity of a T-cell, a NK cell, a macrophage, or a dendritic cell.

37. The method of claim 35, wherein the immune response comprises increasing a cytokine concentration in the patient or inducing maturation of a dendritic cell.

38. The method of claim 37, wherein the cytokine is an interferon or an interleukin.

39. The method of claim 38, wherein the interferon is IFN-α, IFN-β, or IFN-γ.

40. The method of claim 38, wherein the interleukin is IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, or IL-12.

41. The method of claim 18, wherein the MDA-7 polypeptide comprises amino acids from 49 to 206 of SEQ ID NO:2.

42. The method of claim 18, wherein the MDA-7 polypeptide comprises a sequence of SEQ ID NO:2.

43. The method of claim 18, wherein the MDA-7 polypeptide further comprises a secretory signal.

44. The method of claim 43, wherein the secretory signal is further defined as a positively charged N-terminal region in combination with a hydrophobic core.

45. The method of claim 18, wherein the MDA-7 polypeptide is administered systemically to the patient by continuous infusion or by intravenous injection.

46. The method of claim 18, wherein the MDA-7 polypeptide is administered as a direct injection to an immuno-compromised site.

47. The method of claim 18, wherein the immunogenic molecule is Mycobaterium tuberculosis **soluble factor** (Mtb), phenol soluble modulin (PSM), CMV-G, CMV-M, EBV capsid-EB nuclear antigen (EBNA), gp120, gp41, tat, rev, gag, toxa antigen, rubella antigen, mumps antigen, alpha-fetoprotein (AFP), adenocarcinoma antigen (ART-4), BAGE, CAMEL, CAP-I, CASP-8, CDC27m, CDK4/m, CEA, CT, Cyp-B, DAM, ELF2M, ETV6-AMLI, ETS G250, GnT-V, HAGE, HER2/neu, HLA-A*0201-R1701, HPV-E7, HSP 70-2M, HST-2, hTERT, ICE, KIAA 0205, LAGE, LDLR/FUT, MAGE, MART, MC1R, MUCI, MUM-1, MUM-2, MUM-3, NA88-A, NY-ESO-I, p15, Pm1/RARalpha,

PRAME, PSA, PSM, RAGE, RU1, RU2, SAGE, SART-1, SART-3, TEL/AML1, TPI/m, TRP-1, TRP-2, or WT1.

48. The method of claim 48, wherein the nucleic acid sequence encoding the immunogen further comprises an expression vector.

49. A method of treating cancer in a patient comprising providing to the patient a tumor antigen; and administering an effective amount of a MDA-7 polypeptide, wherein the MDA-7 polypeptide provides the patient with a therapeutic benefit.

50. The method of claim 49, wherein the tumor antigen is PSA, CEA, MAGE1, MAGE3, gp100, AFP, her2, tert, muc1, NY-ESO, bcr-abl, trp1, trp2, MART, BAGE, GAGE, or PMSA.

51. A method of treating a tumor in a patient comprising (a) providing to the patient an immunogenic molecule to induce an immune response against the immunogenic molecule; and (b) administering to the patient an effective amount of a MDA-7 polypeptide, wherein the MDA-7 enhances the induced immune response and decreases the tumor as compared to treatment with the immunogenic molecule alone.

52. The method of claim 51, wherein the immunogenic molecule is a tumor antigen.

53. The method of claim 52, wherein the tumor antigen is PSA, CEA, MAGE1, MAGE3, gp100, AFP, her2, tert, muc1, NY-ESO, bcr-abl, trp1, trp2, MART, BAGE, GAGE, or PMSA.

54. The method of claim 51, wherein the decrease is a decrease in tumor size or tumor growth rate.

55. A therapeutic composition comprising a recombinant MDA-7 polypeptide or an isolated nucleic acid encoding the MDA-7 polypeptide and at least one cytokine or an isolated nucleic acid encoding the cytokine.

56. The composition of claim 55, wherein the cytokine is further defined as an interferon α, interferon β, or interferon γ.

57. The composition of claim 56, wherein the cytokine is further defined as interferon γ.

58. The composition of claim 55, wherein an amino acid sequence of the MDA-7 polypeptide is that set forth in SEQ ID NO:2.

59. The composition of claim 55, wherein an amino acid sequence of the MDA-7 polypeptide comprises amino acids 49 to 206 of SEQ ID NO:2.

60. The composition of claim 55, wherein a nucleotide sequence of the nucleic acid encoding an MDA-7 polypeptide is the nucleic acid sequence set forth in SEQ I) NO: 1.

61. A method of enhancing an immune response against an immunogen comprising (a) providing to a patient a polypeptide having an amino acid sequence of the immunogen; and (b) administering to the patient an effective amount of a first composition comprising an MDA-7 polypeptide or a nucleic acid encoding an MDA-7 polypeptide and a second composition comprising an interferon or a nucleic acid encoding the interferon.

62. The method of claim 61, wherein the interferon is interferon α, interferon β, or interferon γ.

63. The method of claim 62, wherein the interferon is interferon γ.

64. The method of claim 61, wherein the first and second compositions

are administered in the same pharmaceutical preparation.

65. The method of claim 61, wherein the first and second compositions are administered in different pharmaceutical preparations.

66. A method of inducing anti-angiogenesis of a tumor in a patient comprising administering to IL-22 receptor-positive cells in the patient an effective amount of an MDA-7 polypeptide to bind the IL-22 receptor and induce anti-angiogenesis of the tumor.

67. The method of claim 66, wherein the 1L-22 receptor-positive cells are endothelial cells.

68. The method of claim 67, wherein the endothelial cells are not adjacent to the tumor.

69. The method of claim 66, wherein the MDA-7 polypeptide is secreted MDA-7 and is glycosylated.

70. A method of inducing cell death in a cell, comprising obtaining an MDA-7 targeting construct, wherein the MDA-7 targeting construct includes a nucleic acid encoding an MDA-7 polypeptide and a targeting sequence under the control of a promoter, and contacting the cell with an amount of the MDA-7 targeting construct that is effective to deliver the MDA-7 targeting construct to the cell, wherein cell death of the cell is induced.

71. The method of claim 70, wherein the targeting construct comprises DNA encoding MDA-7, wherein said DNA does not encode a functional MDA-7 signal peptide.

72. The method of claim 70, wherein the targeting construct comprises DNA encoding MDA-7 and a nuclear localization signal peptide.

73. The method of claim 70, wherein the targeting construct comprises DNA encoding MDA-7 and an endoplasmic reticulum signal peptide.

74. The method of claim 70, wherein the targeting construct comprises DNA encoding MDA-7 and a mitochondrial signal peptide.

75. A method for inducing cell death in a tumor cell comprising administering to the cell i) an MDA-7 polypeptide or a nucleic acid encoding the MDA-7 polypeptide and ii) an **inhibitor** of NF-κB, COX-2, Hsp90, or a protein kinase.

76. A method for inducing cell death in a tumor cell comprising administering to the cell i) an MDA-7 polypeptide or a nucleic acid encoding the MDA-7 polypeptide and ii) an anti-inflammatory agent.

14 ANSWER 3 OF 27 USPATFULL on STN
004:7089 Compositions and methods related to TIM-3, a Th1-specific cell surface molecule.
Kuchroo, Vijay K., Newton, MA, UNITED STATES
Monney, Laurent, Bulle, SWITZERLAND
Gaglia, Jason L., Boston, MA, UNITED STATES
Greenfield, Edward A., Stoughton, MA, UNITED STATES
Freeman, Gordon J., Brookline, MA, UNITED STATES
Waldner, Hanspeter, Cambridge, MA, UNITED STATES
US 2004005322 A1 20040108
APPLICATION: US 2003-354447 A1 20030130 (10)
PRIORITY: US 2002-353107P 20020130 (60)
DOCUMENT TYPE: Utility; APPLICATION.
AS INDEXING IS AVAILABLE FOR THIS PATENT.
B The present invention provides compositions and methods useful for promoting or reducing T-cell trafficking to a target tissue. Also

provided are compositions and methods useful for promoting or **inhibiting** antigen-presenting cell (APC) activation. The invention is related to discovery of functional characteristics of TIM-3, a molecule that is preferentially expressed on the surface of Th1 cells. The methods are useful for treating disorders including cancer, infectious disease, allergy, asthma, and autoimmune disease.

LM What is claimed is:

1. A method for treating a subject in need of an enhanced immune response in a target tissue, comprising administering to the subject a TIM-3-binding molecule in an effective amount to promote T-cell trafficking to the target tissue.

2. The method of claim 1, wherein the TIM-3-binding molecule is an antibody specific for TIM-3.

3. The method of claim 1, wherein the TIM-3-binding molecule is an antibody expressed by hybridoma 8B.2C12.

4. The method of claim 1, wherein the TIM-3-binding molecule is an antibody expressed by hybridoma 25F.1D6.

5. The method of claim 1, wherein the TIM-3-binding molecule is a fragment of an antibody specific for TIM-3.

6. The method of claim 1, wherein the TIM-3-binding molecule binds to an extracellular region of TIM-3.

7. The method of claim 6, wherein the extracellular region of TIM-3 is an IgV domain or a fragment thereof.

8. The method of claim 6, wherein the extracellular region of TIM-3 is a mucin domain or a fragment thereof.

9. The method of claim 1, wherein the subject has or is at risk of having cancer.

10. The method of claim 1, wherein the subject has or is at risk of having an infection.

11. The method of claim 1, wherein the target tissue is selected from the group consisting of: brain, breast, lung, kidney, liver, pancreas, stomach, intestine, ovary, uterus, testis, prostate, marrow, bone, muscle, and skin.

12. The method of claim 1, wherein the target tissue is central nervous system.

13. The method of claim 1, wherein the subject is a human.

14. The method of claim 1, wherein the administering is to a site other than the target tissue.

15. The method of claim 1, wherein the administering is to a site other than a lymph node associated with the target tissue.

16. The method of claim 1, wherein the administering is systemic.

17. The method of claim 1, wherein the administering is intravenous.

18. The method of claim 1, further comprising administering to the subject an adjuvant.

19. The method of claim 1, further comprising administering to the subject an anti-tumor medicament.

20. The method of claim 19, wherein the anti-tumor medicament comprises

a tumor-specific antibody or tumor-specific fragment thereof.

21. The method of claim 1, further comprising administering to the subject a cytokine.

22. The method of claim 1, further comprising administering to the subject an antibacterial medicament.

23. The method of claim 1, further comprising administering to the subject an antiviral medicament.

24. The method of claim 1, further comprising administering to the subject an antifungal medicament.

25. The method of claim 1, further comprising administering to the subject an antiparasitic medicament.

26. A method for treating a subject in need of treatment for a tumor, comprising administering to the subject a TIM-3-binding molecule in an effective amount to promote T-cell trafficking to the tumor.

27. The method of claim 26, wherein the TIM-3-binding molecule is an antibody expressed by hybridoma 8B.2C12.

28. The method of claim 26, wherein the TIM-3-binding molecule is an antibody expressed by hybridoma 25F.1D6.

29. A method for treating a subject in need of treatment for an infection, comprising administering to the subject a TIM-3-binding molecule in an effective amount to promote T-cell trafficking to the infection.

30. The method of claim 29, wherein the TIM-3-binding molecule is an antibody expressed by hybridoma 8B.2C12.

31. The method of claim 29, wherein the TIM-3-binding molecule is an antibody expressed by hybridoma 25F.1D6.

32. A method for reducing T-cell trafficking to a target tissue of a subject, comprising administering to the subject a TIM-3 ligand-binding molecule in an effective amount to reduce T-cell trafficking to a target tissue of the subject.

33. The method of claim 32, wherein the TIM-3 ligand-binding molecule comprises at least one domain of an extracellular region of TIM-3.

34. The method of claim 33, wherein the at least one domain is an IgV domain.

35. The method of claim 32, wherein the TIM-3 ligand-binding molecule is soluble TIM-3.

36. The method of claim 35, wherein the soluble TIM-3 is a fusion protein comprising at least one domain of an extracellular region of TIM-3 and a constant heavy chain or portion thereof of an immunoglobulin.

37. The method of claim 36, wherein the at least one domain is an IgV domain.

38. The method of claim 32, wherein the subject is in need of treatment for an autoimmune disease of the target tissue.

39. The method of claim 32, wherein the target tissue is selected from the group consisting of: central nervous system, pancreatic islets, and joint synovia.

40. The method of claim 38, wherein the autoimmune disease is selected from the group consisting of: multiple sclerosis, type 1 diabetes mellitus, and rheumatoid arthritis.

41. A method for treating or preventing asthma or allergy, comprising increasing activity or expression of TIM-3 in a T cell of a subject to treat or prevent asthma or allergy.

42. The method of claim 41, wherein the T cell is a Th2 cell.

43. A method for treating a Th2-mediated disorder in a subject, comprising expressing TIM-3 on the surface of Th2 cells of a subject having a Th2-mediated disorder in an amount effective to treat the Th2-mediated disorder.

44. The method of claim 43, wherein the Th2-mediated disorder is asthma.

45. A method for promoting antigen-presenting cell (APC) activation, comprising contacting an APC with a TIM-3 ligand-binding molecule in an effective amount to activate the APC.

46. The method of claim 45, wherein the APC is a macrophage.

47. The method of claim 45, wherein the APC is a dendritic cell.

48. The method of claim 45, wherein the TIM-3 ligand-binding molecule comprises an extracellular region of TIM-3.

49. The method of claim 45, wherein the TIM-3 ligand-binding molecule is soluble TIM-3.

50. The method of claim 49, wherein the soluble TIM-3 is a fusion protein comprising at least one domain of an extracellular region of TIM-3 and a constant heavy chain or portion thereof of an immunoglobulin.

51. The method of claim 50, wherein the at least one domain is an IgV domain.

52. The method of claim 49, wherein the method is a method for treating or preventing an intracellular infection.

53. The method of claim 49, wherein the method is a method for treating or preventing cancer.

54. A method for promoting APC activation, comprising contacting a T cell with a TIM-3-binding molecule; and contacting an APC with the T cell to activate the APC.

55. The method of claim 54, wherein the TIM-3-binding molecule is an antibody specific for TIM-3.

56. The method of claim 54, wherein the TIM-3-binding molecule is an antibody expressed by hybridoma 8B.2C12.

57. The method of claim 54, wherein the TIM-3-binding molecule is an antibody expressed by hybridoma 25F.1D6.

58. The method of claim 54, wherein the TIM-3-binding molecule is a fragment of an antibody specific for TIM-3.

59. The method of claim 54, wherein the TIM-3-binding molecule binds to an extracellular region of TIM-3.

60. The method of claim 54, further comprising contacting the T cell

with an antigen specifically bound by a T-cell antigen receptor of the T cell.

61. The method of claim 54, further comprising contacting the APC with an antibody specific for TIM-3.

62. The method of claim 54, wherein the contacting the APC with the T cell is ex vivo.

63. The method of claim 60, wherein the antigen is a tumor antigen.

64. A method for **inhibiting** macrophage activation, comprising contacting an APC with an agent that reduces activity or expression of TIM-3 in an effective amount to **inhibit** activation of the APC.

65. The method of claim claim 64, wherein the agent that reduces activity or expression of TIM-3 is soluble TIM-3.

66. The method of claim 64, wherein the agent that reduces activity or expression of TIM-3 comprises at least one domain of an extracellular region of TIM-3.

67. The method of claim 66, wherein the at least one domain is an IgV domain.

68. The method of claim 64, wherein the agent that reduces activity or expression of TIM-3 is a fusion protein comprising at least one domain of an extracellular region of TIM-3 and a constant heavy chain or portion thereof of an immunoglobulin.

69. The method of claim 68, wherein the at least one domain is an IgV domain.

70. A method for treating or preventing intracellular infections, comprising: promoting macrophage activation by contacting a TIM-3 ligand on the macrophage with a TIM-3 expressing cell.

71. A method for treating or preventing cancer, comprising: promoting APC activation by contacting a TIM-3 ligand on the APC with a TIM-3-expressing cell and contacting the APC with a cancer antigen.

L14 ANSWER 4 OF 27 USPATFULL on STN
2004:7058 Methods for regulating co-stimulatory molecule expression with reactive oxygen.
Rogers, Martha Karen Newell, Colorado Springs, CO, UNITED STATES
Camley, Robert, Colorado Springs, CO, UNITED STATES
Ill, Charles Richard, Carlsbad, CA, UNITED STATES
Christensen, Thomas, Colorado Springs, CO, UNITED STATES
Celinski, Zbigniew, Colorado Springs, CO, UNITED STATES
Trauger, Richard, San Diego, CA, UNITED STATES
Newell, Evan, Toronto, CANADA
Villalobos-Menuey, Elizabeth, Colorado Springs, CO, UNITED STATES
US 2004005291 A1 20040108
APPLICATION: US 2002-272432 A1 20021015 (10)
PRIORITY: US 2001-329477P 20011014 (60)
US 2001-329280P 20011012 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention is based in part on the discovery that the expression of co-stimulatory molecules such as B7.1, B7.2 or CD40 can be regulated using reactive oxygen species (ROS). Thus, the invention relates to methods of regulating co-stimulatory molecules by modulating reactive oxygen. The methods and products are useful, for example, for modulating antigen specific immune responses, treating disease, and for modulating cell growth.

CLM What is claimed is:

1. A method for promoting nerve cell generation, comprising: contacting a nerve cell with a neural cell ROS activator in an effective amount to promote differentiation or growth.

2. The method of claim 1, wherein the neural cell ROS activator is selected from the group consisting of reactive oxygen species, angiostatins, angiogenics, viral components, and exposure to sub-toxic microwaves or low dose radiation.

3. The method of claim 1, further comprising contacting the nerve cell with a neural activating cell.

4. The method of claim 1, wherein the nerve cell is in vitro.

5. The method of claim 1, further comprising maintaining the nerve cell under growth conditions, wherein the conditions include exposure to at least one of nerve growth factor, fibroblast growth factor, and cytokines such as IL-2, IL-4, γ interferon, α, and β interferons, TNF (tumor necrosis factor) α, TGF (T-cell growth factor) α and β, and lymphotoxin.

6. The method of claim 1, further comprising contacting the nerve cell with a receptor for a co-stimulatory molecule.

7. A method for promoting non-neural tissue generation, comprising: contacting a non-neural tissue with an activator of ROS in an effective amount to induce co-stimulatory molecule expression on the surface of cells of the tissue, and exposing the tissue to growth conditions to promote generation of the tissue.

8. The method of claim 7 wherein the ROS activator is selected from the group consisting of γ interferon, lipoproteins, fatty acids, cAMP inducing agents, a UCP expression vector, a B7.1, B7.2 or CD40 expression vector, angiostatins, angiogenics, viral components, and exposure to sub-toxic microwaves or low dose radiation.

9. The method of claim 7, further comprising exposing the non-neural tissue to a T cell.

10. The method of claim 9, wherein the non-neural tissue is exposed to the T cell in vitro.

11. The method of claim 9, wherein the non-neural tissue is implanted in a subject after exposure to the T cell.

12. The method of claim 11, wherein the T cell is a cell of the subject.

13. The method of claim 12, wherein the non-neural tissue is autologous tissue.

14. The method of claim 12, wherein the non-neural tissue is a donor organ.

15. The method of claim 7, wherein a biopsy of the non-neural tissue is removed from a subject and wherein the biopsy of non-neural tissue is exposed to a T cell of the subject.

16. The method of claim 7, wherein the growth conditions include exposure to at least one of insulin, fibroblast growth factor, platelet derived growth factor, erythropoietin, and cytokines such as IL-2, IL-4, γ interferon, α and β, interferons, TNF (tumor necrosis factor) α, TGF (T-cell growth factor) α and β, and lymphotoxin.

17. A method for transplanting an organ into a recipient subject, comprising, treating a donor organ with an **inhibitor** of ROS in an effective amount to reduce costimulatory molecule expression on cells of the donor organ, and transplanting the donor organ into the recipient subject.

18. The method of claim 17, wherein the **inhibitor** of ROS is selected from the group consisting of compounds which activate or induce glutathione S reductase, glutathione, Copper/Zinc superoxide dismutase, and Manganese superoxide dismutase.

19. A method for treating cancer, comprising: exposing cancer cells of a subject to sub-toxic levels of microwave or to sub-toxic levels H_{202} in an effective amount to induce expression of a co-stimulatory molecule on the surface of the cancer cells and contacting the cell with an agent to kill the cell in order to treat the cancer.

20. The method of claim 19, further comprising exposing the cancer cells to 2-deoxyglucose or analogs thereof.

21. The method of claim 19, wherein the agent is a co-stimulatory molecule receptor.

22. The method of claim 21, wherein the co-stimulatory molecule receptor is on an immune cell.

23. The method of claim 21, wherein the co-stimulatory molecule receptor is a soluble receptor.

24. A method for **inhibiting** co-stimulatory molecule expression in a cell for in vivo transplantation, comprising: contacting a cell with an **inhibitor** of ROS to **inhibit** co-stimulatory molecule expression in the cell, and implanting the cell in a subject.

25. The method of claim 24, wherein the cell is a stem cell.

26. The method of claim 24, wherein the co-stimulatory molecule is B7.1, B7.2 or CD40.

27. The method of claim 24, wherein the cell is selected from the group of cells consisting of kidney, lung, pancreas, skin, liver, eye, ovary, testes, and Sertoli cells.

28. The method of claim 24, further comprising administering the stem cell to a subject.

29. The method of claim 24, wherein the cell is grown in vitro under growth conditions prior to implantation.

30. The method of claim 28 wherein the **inhibitor** of reactive oxygen species is a compound selected from the group consisting of glutathione S reductase, glutathione, Copper/Zinc superoxide dismutase, and Manganese superoxide dismutase

31. A method for inducing co-stimulatory molecule expression in a growth induced cell, comprising: contacting a cell with an activator of ROS to induce co-stimulatory molecule expression in the cell, and exposing the cell to growth conditions to promote cell proliferation.

32. The method of claim 31, wherein the growth conditions include exposure to at least one of insulin, nerve growth factor, fibroblast growth factor, platelet derived growth factor, erythropoietin, and cytokines such as IL-2, IL-4, γ interferon, α and β interferons, TNF (tumor necrosis factor) α, TGF (T-cell growth factor) α and β, and lymphotoxin.

33. The method of claim 31, wherein the co-stimulatory molecule is B7.1, B7.2 or CD40.

34. The method of claim 31, wherein the method is performed in vitro.

35. The method of claim 31, wherein the activator of ROS a reactive oxygen species.

36. The method of claim 34, further comprising administering the cell to a subject.

37. The method of claim 31, further comprising contacting the cell with an antigen.

38. The method of claim 31, wherein the method is performed in vivo in a subject.

39. The method of claim 31, wherein the activator of ROS is an **inhibitor** of mitochondrial electron transport selected from the group consisting of reactive oxygen species, angiostatins, angiogenics, viral components, and exposure to sub-toxic microwaves or low dose radiation.

40. The method of claim 39, wherein the viral component is a gene product selected from the group consisting of **HIV** Nef, **HIV** tat, and adenoviral E1B.

41. The method of claim 31 wherein the activator of ROS is an **inhibitor** of glutathione or glutathione S reductase.

42. The method of claim 31, wherein the activator of ROS is an **inhibitor** of superoxide dismutase.

43. The method of claim 31, wherein the activator of ROS is an **inhibitor** of lysosomal UCP.

44. The method of claim 31, wherein the activator of ROS is exposure to microwaves.

45. The method of claim 43, wherein the cell is a nerve cell.

46. The method of claim 31, wherein the cell is a neutrophil.

47. A method for modulating B7.1, B7.2 or CD40 expression on embryonic stem cells, comprising: contacting an embryonic stem cell with a compound for modulating reactive oxygen species to modulate B7.1, B7.2 or CD40 expression on the embryonic stem cell.

48. The method of claim 47, wherein the compound for modulating reactive oxygen species is an **inhibitor** of ROS.

49. The method of claim 47, wherein the compound for modulating reactive oxygen species is a reactive oxygen species or an activator of ROS.

50. The method of claim 47, further comprising administering the embryonic stem cell to a subject.

51. A method for treating autoimmune disease, comprising, administering to a subject having or at risk of developing an autoimmune disease an **inhibitor** of ROS in an effective amount to reduce costimulatory molecule expression on target autoimmune cells in order to treat the autoimmune disease.

52. The method of claim 51, wherein the **inhibitor** of ROS is selected from the group consisting of compounds which activate or induce glutathione S reductase, glutathione, Copper/Zinc superoxide dismutase,

or Manganese superoxide dismutase.

53. The method of claim 51, wherein the autoimmune disease is multiple sclerosis.

L14 ANSWER 5 OF 27 USPATFULL on STN
2003:332338 Generation of antigen specific T **suppressor** cells for treatment of rejection.
Suciu-Foca, Nicole, Cliffside Park, NJ, United States
The Trustees of Columbia University, New York, NY, United States (U.S. corporation)
US 6667175 B1 20031223
APPLICATION: US 1999-333809 19990615 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides a method of generating antigen specific allospecific human **suppressor** **CD8**+CD28- T cells. This invention also provides a method of generating xenospecific human **suppressor** **CD8**+CD28- T cells. This invention further provides a method of generating allopeptide antigen specific human **suppressor** **CD8**+CD28- T cells. Methods of treatment for reduction of risk of rejection of allografts and xenografts and autoimmune diseases using the human **suppressor** **CD8**+CD28- T cells so produced are also provides, as are methods of preventing rejection and autoimmune diseases, and vaccines comprising the produced **suppressor** T cells. Methods of diagnosis to determine whether a level of immuno-**suppressant** therapy requires a reduction are provided.
CLM What is claimed is:
1. A method of generating allopeptide antigen specific human **suppressor** **CD8**+CD28- T cells which comprises: a) obtaining peripheral blood T cells from a subject; b) stimulating by multiple priming a T cell line from the T cells obtained in step (a) with autologous antigen presenting cells (APCs) pulsed with an allopeptide, said allopeptide comprising an amino acid sequence comprising both MHC class I and MHC class II amino acid sequences wherein the amino acid sequences are binding sequences and are recognized by the primed T cell line; c) isolating primed populations of **CD8+ T cells** and of CD4+ T helper cells from the T cell line stimulated in step (b); d) isolating primed **CD8**+CD28- T cells from the isolated primed **CD8+ T cells** of step (c); e) detecting **suppression** by the primed **CD8**+CD28- T cells isolated in step (d) of interaction between the CD4+ T helper cells isolated in step (c) and autologous antigen presenting cells (APCs) expressing the same MHC class I and MHC class II binding motifs as expressed by the APCs used to stimulate the T cell line of step (b), thereby identifying allopeptide antigen specific human **suppressor** **CD8**+CD28- T cells; and f) expanding the allopeptide antigen specific human **suppressor** **CD8**+CD28- T cells identified in step (e), thereby generating allopeptide antigen specific human **suppressor** **CD8**+CD28- T cells.

2. The method of claim 1 wherein the allopeptide is a peptide antigen or a whole protein antigen.

3. The method of claim 2, wherein the peptide antigen is tat-DR4 (SEQ ID NO:1).

4. The method of claim 2, wherein the peptide antigen is a peptide comprising an amino acid sequence of a hypervariable region of HLA-DR B1.

L14 ANSWER 6 OF 27 USPATFULL on STN
2003:294272 Non-stochastic generation of genetic vaccines.
Short, Jay M., Rancho Santa Fe, CA, UNITED STATES
US 2003207287 A1 20031106

APPLICATION: US 2002-223507 A1 20020819 (10)
PRIORITY: US 1995-8311P 19951207 (60)
US 1995-8316P 19951207 (60)
DOCUMENT TYPE: Utility; APPLICATION.
S INDEXING IS AVAILABLE FOR THIS PATENT.

This invention provides methods of obtaining vaccines by use of non-stochastic methods of directed evolution (DirectEvolution.TM.). These methods include non-stochastic polynucleotide site-saturation mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). Through use of the claimed methods, vectors can be obtained which exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like.

M What is claimed is:

1. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response, or encodes a polypeptide that has an optimized modulatory effect on an immune response, the method comprising: creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; wherein optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation and iterative manner; whereby these directed evolution methods include the introduction of mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

2. The method of claim 1, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector. Screening

3. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response, or encodes a polypeptide that has an optimized modulatory effect on an immune response, the method comprising: screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created.

4. The method of claim 3, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector. Evolution & Screening

5. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response, or encodes a polypeptide that has an optimized modulatory effect on an immune response, the method comprising: a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response induced by a genetic vaccine vector; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative

manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

6. The method of claim 5, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

7. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide is incorporated into a genetic vaccine vector.

8. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide, or a polypeptide encoded by the optimized non-stochastically generated polynucleotide, is administered in conjunction with a genetic vaccine vector.

9. The method of any of claims 1-6, wherein the library of non-stochastically generated progeny polynucleotides is created by a process selected from the group consisting of gene reassembly, oligonucleotide-directed saturation mutagenesis, and any combination, permutation and iterative manner.

10. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide that has a modulatory effect on an immune response is obtained by: a) non-stochastically reassembling at least two parental template polynucleotide, each of which is, or encodes a molecule that is, involved in modulating an immune response; wherein the first and second parental templates differ from each other in two or more nucleotides, to produce a library of non-stochastically generated polynucleotides; and b) screening the library to identify at least one optimized non-stochastically generated polynucleotide that exhibits, either by itself or through the encoded molecule, an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created.

11. The method of claim 10, wherein the method further comprises the steps of: c) subjecting a working optimized non-stochastically generated polynucleotide to a further round of non-stochastic reassembly with at least one additional polynucleotide, which is the same or different from the first and second polynucleotides, to produce a further working library of recombinant polynucleotides; d) screening the further working library to identify at least one further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created; and e) optionally repeating c) and d) as necessary, until a desirable further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response than a form of the nucleic acid from which the library was created.

12. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that can interact with a cellular receptor involved in mediating an immune response; wherein the polypeptide acts as an agonist or antagonist of the receptor.

13. The method of claim 12, wherein the cellular receptor is a macrophage scavenger receptor.

14. The method of claim 12, wherein the cellular receptor is selected from the group consisting of a cytokine receptor and a chemokine

receptor.

15. The method of claim 14, wherein the chemokine receptor is CCR6.

16. The method of claim 12, wherein the polypeptide mimics the activity of a natural ligand for the receptor but does not induce immune reactivity to said natural ligand.

17. The method of claim 12, wherein the library is screened by: i) expressing the non-stochastically generated progeny polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package; ii) contacting the replicable genetic packages with a plurality of cells that display the receptor; and iii) identifying cells that exhibit a modulation of an immune response mediated by the receptor.

18. The method of claim 17, wherein the replicable genetic package is selected from the group consisting of a bacteriophage, a cell, a spore, and a virus.

19. The method of claim 18, wherein the replicable genetic package is an M13 bacteriophage and the protein is encoded by geneIII or geneVIII.

20. The method of claim 12, which method further comprises introducing the optimized non-stochastically generated polynucleotide into a genetic vaccine vector and administering the vector to a mammal, wherein the peptide or polypeptide is expressed and acts as an agonist or antagonist of the receptor.

21. The method of claim 12, which method further comprises producing the polypeptide encoded by the optimized non-stochastically generated polynucleotide and introducing the polypeptide into a mammal in conjunction with a genetic vaccine vector.

22. The method of claim 12, wherein the optimized non-stochastically generated polynucleotide is inserted into an antigen-encoding nucleotide sequence of a genetic vaccine vector.

23. The method of claim 22, wherein the optimized non-stochastically generated polypeptide is introduced into a nucleotide sequence that encodes an M-loop of an HBsAg polypeptide.

24. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide comprises a nucleotide sequence rich in unmethylated CpG.

25. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that **inhibits** an allergic reaction.

26. The method of claim 25, wherein the polypeptide is selected from the group consisting of interferon-α, interferon-γ, IL-10, IL-12, an antagonist of IL-4, an antagonist of IL-5, and an antagonist of IL-13.

27. The method of 1, wherein the optimized recombinant polynucleotide encodes an antagonist of IL-10.

28. The method of claim 27, wherein the antagonist of IL-10 is soluble or defective IL-10 receptor or IL-20/MDA-7.

29. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a co-stimulator.

30. The method of claim 29, wherein the co-stimulator is B7-1 (**CD80**) or B7-2 (**CD86**) and the screening step involves selecting variants with

altered activity through CD28 or CTLA-4.

31. The method of claim 29, wherein the co-stimulator is CD1, CD40, CD154 (ligand for CD40) or CD150 (SLAM).

32. The method of claim 29, wherein the co-stimulator is a cytokine.

33. The method of claim 32, wherein the cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF-α, IFN-α, IFN-γ, and IL-20 (MDA-7).

34. The method of 33, wherein the library of non-stochastically generated polynucleotides is screened by testing the ability of cytokines encoded by the non-stochastically generated polynucleotides to activate cells which contain a receptor for the cytokine.

35. The method of claim 34, wherein the cells contain a heterologous nucleic acid that encodes the receptor for the cytokine.

36. The method of 33, wherein the cytokine is interleukin-12 and the screening is performed by: growing mammalian cells which contain the genetic vaccine vector in a culture medium; and detecting whether T cell proliferation or T cell differentiation is induced by contact with the culture medium.

37. The method of 33, wherein the cytokine is interferon-α and the screening is performed by: i) expressing the non-stochastically generated polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package; ii) contacting the replicable genetic packages with a plurality of B cells; and iii) identifying phage library members that are capable of **inhibiting** proliferation of the B cells.

38. The method of claim 33, wherein the immune response of interest is differentiation of T cells to T_{H1} cells and the screening is performed by contacting a population of T cells with the cytokines encoded by the members of the library of recombinant polynucleotides and identifying library members that encode a cytokine that induces the T cells to produce IL-2 and interferon-γ.

39. The method of claim 32, wherein the cytokine encoded by the optimized non-stochastically generated polynucleotide exhibits reduced immunogenicity compared to a cytokine encoded by a non-optimized polynucleotide, and the reduced immunogenicity is detected by introducing a cytokine encoded by the non-stochastically generated polynucleotide into a mammal and determining whether an immune response is induced against the cytokine.

40. The method of claim 29, wherein the co-stimulator is B7-1 (**CD80**) or B7-2 (**CD86**) and the cell is tested for ability to costimulate an immune response.

41. The method of any of claims 1-6, wherein the optimized recombinant polynucleotide encodes a cytokine antagonist.

42. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of a soluble cytokine receptor and a transmembrane cytokine receptor having a defective signal sequence.

43. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of ΔIL-1 OR and ΔIL-4R.

44. The method of any of claims 1-6, wherein the optimized

non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H1} immune response.

45. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H2} immune response. Decreased Immune Response

46. The method of any of claims 1-6, wherein said optimized modulatory effect on an immune response is a decrease in an unwanted modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having a decreased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having decreased antigenicity with respect to at least one host recipient of said molecule. Increased Immune Response

47. The method of any of claims 1-6, wherein said optimized modulatory effect on an immune response is an increase in a desirable modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having an increased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having increased antigenicity with respect to at least one host recipient of said molecule. Decreased and Increased Immune Response

48. The method of any of claims 1-6, wherein said optimized modulatory effect on an immune response is both a decrease in a first unwanted modulatory effect on an immune response as well as an increase in a second desirable modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having both a decreased ability to elicit a first immune response from a first host recipient of said molecule as well as a an increased ability to elicit a second immune response from a second host recipient of said molecule; whereby the first and the second recipient hosts can be the same or different; whereby each of the first and the second recipient hosts can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having both a first decreased antigenicity with respect to at least one host recipient of said molecule and a second decreased antigenicity with respect to at least one host recipient of said molecule.

49. The method of claim 48, wherein said first and said second modulatory effect on an immune response are evolved for respectively a first and a second module on the same multimodule vaccine vector; whereby a module is exemplified by the following modules, as well as by a fragment derivative or analog thereof: an antigen coding sequence, a polyadenylation sequence, a sequence coding for a co-stimulatory molecule, a sequence coding for an inducible repressor or transactivator, a eukaryotic origin or replication, a prokaryotic origin of replication, a sequence coding for a prokaryotic marker,, and enhancer, a promoter, and operator, and an intron. Stability

50. The method of any of claims 1-6, wherein the optimized modulatory effect on an immune response is comprised of an increase in the stability of the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; whereby application of the method can be used to generate a molecule having an increased stability ex vivo, thus, for example, increasing shelf-life and/or ease of storage and/or length of time before expiration of activity upon storage; and whereby application of the method can also be used to generate a molecule having an increased stability in vivo upon administration to a host recipient, thus, for example, increasing resistance to digestive acids and/or increasing stability in the circulation and/or any other method of

elimination or destruction by the host recipient. Human Vaccines

50. The method of any of claims 1-6, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in a human host recipient; whereby application of the method can thus be used to generate an optimized genetic vaccine for human recipeints. Animal Vaccines

51. The method of any of claims 1-6, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in an animal host recipient; whereby application of the method can thus be used to generate an optimized genetic vaccine for animal recipients, including animals that are farmed or raised by man, animals that are not farmed or raised by man, domesticated animals, and non-domesticated animals. Accessory Molecules

52. A method for obtaining an optimized polynucleotide that encodes an accessory molecule that improves the transport or presentation of antigens by a cell, the method comprising: a) creating a library of non-stochastically generated polynucleotides by subjecting to optimization by non-stochastic directed evolution a parental polynucleotide set in which is encoded all or part of the accessory molecule; and b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that encodes a recombinant molecule that confers upon a cell an increased or decreased ability to transport or present an antigen on a surface of the cell compared to an accessory molecule encoded by template polynucleotides not subjected to the non-stochastic reassembly; whereby application of the method can thus be used to generate an optimized molecule for human recipients &/or animal recipients, including animals that are farmed or raised by man, animals that are not farmed or raised by man, domesticated animals, and non-domesticated animals; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

53. The method of claim 52, wherein the screening involves: i) introducing the library of non-stochastically generated polynucleotides into a genetic vaccine vector that encodes an antigen to form a library of vectors; introducing the library of vectors into mammalian cells; and ii) identifying mammalian cells that exhibit increased or decreased immunogenicity to the antigen.

54. The method of claim 52, wherein the accessory molecule comprises a proteasome or a TAP polypeptide.

55. The method of claim 52, wherein the accessory molecule comprises a cytotoxic T-cell inducing sequence.

56. The method of claim 55, wherein the cytotoxic T-cell inducing sequence is obtained from a hepatitis B surface antigen.

57. The method of claim 52, wherein the accessory molecule comprises an immunogenic agonist sequence. Plant Expression

58. A method for obtaining an immunomodulatory polynucleotide that has, an optimized expression in a recombinant expression host, the method comprising: creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; whereby

optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation and iterative manner; whereby these directed evolution methods include the introduction of mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

59. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising: screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host when compared to the expression of a parental polynucleotide from which the library was created.

60. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising: a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and b) screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host when compared to the expression of a parental polynucleotide from which the library was created; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

61. The method of any of claims 58-60, wherein the recombinant expression host is a prokaryote.

62. The method of any of claims 58-60, wherein the recombinant expression host is a eukaryote.

63. The method of claim 62, wherein the recombinant expression host is a plant.

64. The method of any of claims 63, wherein the recombinant expression host is a monocot.

65. The method of any of claims 63, wherein the recombinant expression host is a dicot.

66. The method of any of claims 1-6, 52, or 58-60, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "gene site saturation mutagenesis" as described herein.

67. The method of any of claims 1-6, 52, or 58-60, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "synthetic ligation polynucleotide reassembly" as described herein.

68. The method of any of claims 1-6, 52, or 58-60, wherein creating a library of non-stochastically generated progeny polynucleotides from a

parental polynucleotide set is comprised of subjecting the parental polynucleotide set to both "gene site saturation mutagenesis" as described herein, and to "synthetic ligation polynucleotide reassembly" as described herein.

L14 ANSWER 7 OF 27 USPATFULL on STN
2003:226589 Nucleic acids encoding CD100 molecules.
Hall, Kathryn T., Boston, MA, UNITED STATES
Freeman, Gordon J., Brookline, MA, UNITED STATES
Schultze, Joachim L., Brookline, MA, UNITED STATES
Boussiotis, Vassiliki A., Brookline, MA, UNITED STATES
Nadler, Lee M., Newton, MA, UNITED STATES
Dana-Farber Cancer Institute (U.S. corporation)
US 2003158402 A1 20030821
APPLICATION: US 2002-320769 A1 20021216 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Isolated nucleic acid molecules encoding novel CD100 molecules which stimulate a leukocyte response, such as a B cell response, including B cell aggregation, B cell differentiation, B cell survival, and/or T cell proliferationare disclosed. These novel molecules have a certain homology to semaphorins, proteins which are growth cone guidance molecules that are critical for guiding growing axons of neurons to their targets. In addition to isolated nucleic acids molecules, antisense nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced are also described. The invention further provides isolated CD100 proteins, fusion proteins and active fragments thereof. Diagnostic and therapeutic methods utilizing compositions of the invention are also provided.
CLM What is claimed is:
1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a CD100 antigen.

2. The isolated nucleic acid molecule of claim 1, which is a cDNA.

3. The isolated nucleic acid molecule of claim 2, wherein the CD100 antigen is human.

4. The isolated nucleic acid of claim 3 comprising a nucleotide sequence shown in FIG. 1, SEQ ID NO: 1.

5. The isolated nucleic acid of claim 4 comprising the coding region.

6. The isolated nucleic acid of claim 3 derived from a hematopoietic cell which hybridizes under high stringency conditions to a nucleic acid molecule comprising a nucleotide sequence shown in FIG. 1, SEQ ID NO: 1.

7. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein wherein the protein comprises an amino acid sequence having at least about 80% overall amino acid sequence identity with an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

8. The isolated nucleic acid molecule of claim 7, wherein the protein has a CD100 activity.

9. An isolated nucleic acid molecule encoding an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

10. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein, wherein the protein comprises a semaphorin domain having an amino acid sequence at least 80% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

11. An isolated nucleic acid molecule comprising a nucleotide sequence

encoding a protein, wherein the protein comprises a) a semaphorin domain having an amino acid sequence at least 60% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2 ; and b) an immunoglobulin-like domain having an amino acid sequence at least 50% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

12. The isolated nucleic acid molecule of claim 11, further comprising c) a cytoplasmic domain having an amino acid sequence at least 50% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

13. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein, wherein the protein comprises an extracellular domain having an amino acid sequence at least 80% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

14. An isolated nucleic acid molecule encoding a CD100 fusion protein comprising a nucleotide sequence encoding a first peptide having an amino acid sequence at least 80% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2 and a nucleic sequence encoding a second peptide corresponding to a moiety that alters the solubility, binding affinity or valency of the first peptide.

15. The isolated nucleic acid molecule of claim 14, wherein the first peptide comprises an extracellular domain of a human CD100 antigen.

16. The isolated nucleic acid molecule of claim 14, wherein the first peptide comprises a semaphorin domain of a human CD100 antigen.

17. The isolated nucleic acid molecule of claim 14, wherein the second peptide comprises an immunoglobulin constant region.

18. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a peptide comprising a fragment of at least about 30 amino acids of the sequence shown in FIG. 2, SEQ ID NO: 2.

19. The isolated nucleic acid molecule of claim 18, wherein the peptide has a CD100 activity.

20. An isolated nucleic acid molecule of claim 1, which is antisense to the nucleic acid molecule of claim 1.

21. An isolated nucleic acid molecule of claim 1, which is antisense to the nucleic acid molecule of claim 4.

31. An isolated nucleic acid molecule of claim 1, which is antisense to the nucleic acid molecule of claim 5.

32. A vector comprising a nucleotide sequence encoding a CD100 antigen.

33. A vector comprising a nucleotide sequence encoding a protein comprising an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

34. A host cell comprising the vector of claim 32.

35. A host cell comprising the vector of claim 33.

36. A method for producing a CD100 antigen comprising culturing a host cell of claim 34 in a suitable medium such that the CD100 antigen is produced.

37. A method for producing a CD100 antigen comprising culturing a host cell of claim 35 in a suitable medium such that the CD100 antigen is produced.

38. An isolated protein having a CD100 activity.

39. The isolated protein of claim 38, which is human.

40. The isolated protein of claim 39, wherein the protein comprises an amino acid sequence having at least about 80% overall amino acid sequence identity with an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

41. The isolated protein of claim 40, wherein the protein comprises an amino acid sequence having at least about 90% overall amino acid sequence identity with an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

42. The isolated protein of claim 40, wherein the protein comprises an amino acid sequence having at least about 95% overall amino acid sequence identity with an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

43. The isolated protein of claim 39, wherein the protein has a CD100 activity.

44. The isolated protein of claim 43, which comprises an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

45. An isolated protein comprising a semaphorin domain having an amino acid sequence at least 80% identical to an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

46. The isolated protein of claim 45 having an amino acid sequence at least 90% identical to an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

47. An isolated protein comprising a) a semaphorin domain having an amino acid sequence at least 60% identical to an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2); and b) an immunoglobulin-like domain having an amino acid sequence that is at least 50% identical to an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

48. The isolated protein of claim 47, further comprising c) a cytoplasmic domain having an amino acid sequence at least 50% identical to an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

49. An isolated protein comprising an extracellular domain having an amino acid sequence at least 80% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

50. An CD100 fusion protein comprising a first peptide having an amino acid sequence at least 80% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2 and a second peptide corresponding to a moiety that alters the solubility, binding affinity or valency of the first peptide.

51. The fusion protein of claim 50, wherein the first peptide comprises an extracellular domain of a human CD100 antigen.

52. The fusion protein of claim 50, wherein the first peptide comprises a semaphorin domain of a human CD100 antigen.

53. The fusion protein of claim 50, wherein the second peptide comprises an immunoglobulin constant region.

54. A peptide comprising a fragment of at least about 30 amino acids of the sequence shown in FIG. 2, SEQ ID NO: 2.

55. The peptide of claim 54, which has a CD100 activity.

56. The peptide of claim 54, wherein the fragment is at least about 40 amino acids in length.

57. The peptide of claim 56, which has a CD100 activity.

58. The peptide of claim 56, wherein the fragment is at least about 50 amino acid in length.

59. The peptide of claim 58, which has a CD100 activity.

60. A pharmaceutical composition comprising the protein of claim 44 and a pharmaceutically acceptable carrier.

61. A pharmaceutical composition comprising the protein of claim 50 and a pharmaceutically acceptable carrier.

62. A pharmaceutical composition comprising the protein of claim 55 and a pharmaceutically acceptable carrier.

63. A vaccine composition comprising at least one antigen and a first agent which stimulates a CD100 ligand-associated signal.

64. The composition of claim 63, further comprising a second agent which stimulates a CD40-associated signal.

65. A method for modulating a B cell response comprising contacting the B cell with an agent which modulates a CD100 ligand-associated signal in the B cell, such that a response by the B cell is modulated.

66. The method of claim 65, wherein the agent stimulates a CD100 ligand associated signal, such that a response by the B cell is stimulated.

67. The method of claim 66, wherein the agent is a stimulatory form of a CD100 antigen.

68. The method of claim 67, wherein the stimulatory form of a CD100 antigen is a soluble form of a CD100 antigen.

69. The method of claim 68, wherein the soluble form of a CD100 antigen is an immunoglobulin fusion protein.

70. The method of claim 67, wherein the stimulatory form of a CD100 antigen is attached to a solid phase support.

71. The method of claim 68, wherein the solid phase support is a cell membrane.

72. The method of claim 66, further comprising contacting the B cell with a second agent which provides a stimulatory signal to the B cell.

73. The method of claim 72, wherein the second agent stimulates a CD40 associated signal in the B cell.

74. The method of claim 73, wherein the second agent is a CD40 ligand.

75. The method of claim 66, further comprising contacting the B cells with T cells.

76. The method of claim 75, further comprising contacting the B cells with at least one antigen.

77. The method of claim 65, wherein the agent **inhibits** a CD100 ligand-associated signal, such that a response by the B cell is **inhibited**.

78. The method of claim 77, wherein the agent interacts with CD100.

79. The method of claim 78, wherein the agent is an antibody to CD100.

80. The method of claim 77, wherein the agent interacts with a CD100 ligand.

81. The method of claim 80, wherein the agent is an **inhibitory** form of CD100.

82. The method of claim 65, wherein the B cell response is B cell aggregation.

83. The method of claim 65, wherein the B cell response is B cell differentiation.

84. The method of claim 83, wherein the B cells differentiate into plasma cells.

85. The method of claim 83, wherein the B cells differentiate to memory B cells.

86. The method of claim 65, wherein the B cell response is B cell viability.

87. The method of claim 65, wherein contacting the cells is in a subject, such that a response by the B cell is modulated in the subject.

88. The method of claim 87, wherein the agent stimulates a B cell response in a subject infected with a pathogen, such that elimination of the pathogen by the subject is enhanced.

89. The method of claim 87, wherein the agent stimulates a B cell response in a subject having a low grade lymphoma, such that the B cell differentiation in the subject is stimulated.

90. The method of claim 88, further comprising administering to the subject a second agent which stimulates a CD40-associated signal in the B cell.

91. The method of claim 87, wherein the agent **inhibits** a B cell response in an allergic subject, such that the B cell response by the subject to an allergen is **inhibited**.

92. The method of claim 87, wherein the agent **inhibits** a B cell response in a subject having a large cell lymphoma, such that differentiation of the B cell in the subject is **inhibited**.

93. A method for modulating an interaction between an immune cell and a nerve cell in a subject, comprising administering to a subject an agent which modulates a CD100 ligand-associated signal in the nerve cell, such that modulation of the interaction between the immune cell and the nerve cell in the subject occurs.

94. A method for modulating axonal growth of a neuron, comprising contacting the neuron with a modulating form of CD100, such that axonal growth is modulated.

95. A method for modulating a T cell response, comprising contacting the T cell with an agent which modulates a CD100 ligand-induced signal in the T cell, such that a response in the T cell is modulated.

96. The method of claim 95, wherein a T cell is contacted with an agent which stimulates a CD100 ligand-induced signal in the T cell, such that a response in the T cell is stimulated.

97. The method of claim 96, further comprising contacting the T cell with an agent which provides a primary activation signal to the T cell.

98. The method of claim 95, wherein the T cell is contacted with an agent which **inhibits** a CD100 ligand-induced signal in the T cell, such that a response in the T cell is **inhibited**.

4 ANSWER 8 OF 27 USPATFULL on STN
03:140127 BAFF, **inhibitors** thereof and their use in the modulation of B-cell response and treatment of autoimmune disorders.
MacKay, Fabienne, Vaucluse, AUSTRALIA
Kalled, Susan, Concord, MA, UNITED STATES
US 2003095967 A1 20030522
APPLICATION: US 2001-45574 A1 20011107 (10)
PRIORITY: US 1999-117169P 19990125 (60)
US 1999-143228P 19990709 (60)
DOCUMENT TYPE: Utility; APPLICATION.
AS INDEXING IS AVAILABLE FOR THIS PATENT.
The invention provides methods for treating or preventing disorders associated with expression of BAFF comprising BAFF and fragments thereof, antibodies, agonists and antagonists.
LM What is claimed is:
1. A method of stimulating B-cell growth in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; and (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule.

2. A method of stimulating immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule.

3. A method of co-stimulating B-cell growth and immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; and (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule.

4. A method of stimulating dendritic cell-induced B-cell growth and maturation comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; and (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule.

5. The method according to claims 1-4 wherein the BAFF ligand is a soluble BAFF ligand.

6. The method according to claim 5 wherein the soluble BAFF ligand is a recombinant BAFF ligand.

7. The method according to claims 1-4 wherein the anti-CD40 molecule is a monoclonal antibody.

8. The method according to claims 1-4 wherein the animal is of mammalian origin.

9. The method according to claim 8 wherein the mammal is human.

10. A method of **inhibiting** B-cell growth in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a anti-BAFF ligand molecule or an active fragment thereof; (b) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (c) an antibody specific for BAFF ligand or an active fragment thereof; and (d) an antibody specific for BAFF ligand receptor or an epitope thereof.

11. A method of **inhibiting** immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a anti-BAFF ligand molecule or an active fragment thereof; (b) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (c) an antibody specific for BAFF ligand or an active fragment thereof; and (d) an antibody specific for BAFF ligand receptor or an epitope thereof.

12. A method of co-**inhibiting** B-cell growth and immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a anti-BAFF ligand molecule or an active fragment thereof; (b) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (c) an antibody specific for BAFF ligand or an active fragment thereof; and (d) an antibody specific for BAFF ligand receptor or an epitope thereof.

13. A method of **inhibiting** dendritic cell-induced B-cell growth and maturation in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a anti-BAFF ligand molecule or an active fragment thereof; (b) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (c) an antibody specific for BAFF ligand or an active fragment thereof; and (d) an antibody specific for BAFF ligand receptor or an epitope thereof.

14. The method according to claims 10-13, wherein the anti-BAFF ligand is soluble.

15. The method according to claim 14, wherein the soluble anti-BAFF ligand is a recombinant anti-BAFF ligand.

16. The method according to claims 10-13, wherein the anti-BAFF antibody is a monoclonal antibody. The method according to claims 10-13, wherein the anti-BAFF receptor antibody is a monoclonal antibody.

17. A method of treatment of an autoimmune disease comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule; (e) a anti-BAFF ligand molecule or an active fragment thereof; (f) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (g) an antibody specific for BAFF ligand or an active fragment thereof; and (h) an antibody specific for BAFF ligand receptor or an epitope thereof.

18. A method of treating a disorder related to BAFF-ligand comprising the steps of: (a) introducing into a desired cell a therapeutically effective amount of a vector containing a gene encoding for a BAFF-related molecule; and (b) expressing said gene in said cell.

19. The method according to claim 18, wherein the BAFF-related molecule is selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment

thereof and a CD40 ligand; (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule; (e) a anti-BAFF ligand molecule or an active fragment thereof, (f) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (g) an antibody specific for BAFF ligand or an active fragment thereof; and (h) an antibody specific for BAFF ligand receptor or an epitope thereof.

20. The method according to claims 17-19, wherein the BAFF ligand is a soluble BAFF ligand.

21. The method according to claim 20, wherein the soluble BAFF ligand is a recombinant BAFF ligand.

22. The method according to claims 17-19, wherein the anti-CD40 molecule is a monoclonal antibody.

23. The method according to claims 17-19, wherein the anti-BAFF ligand is soluble.

24. The method according to claim 23, wherein the soluble anti-BAFF ligand is a recombinant anti-BAFF ligand.

25. The method according to claims 17-19, wherein the anti-BAFF antibody is a monoclonal antibody.

26. The method according to claims 17-19, wherein the anti-BAFF receptor antibody is a monoclonal antibody.

27. A method of inducing cell death comprising the administration of an agent capable of interfering with the binding of a BAFF-ligand to a receptor.

28. A method of treating, **suppressing** or altering an immune response involving a signaling pathway between a BAFF-ligand and its receptor comprising the step of administering an effective amount of an agent capable of interfering with the association between the BAFF-ligand and its receptor.

29. A method of **inhibiting** inflammation comprising the step of administering a therapeutically effective amount of an antibody specific for a BAFF-ligand or an active fragment thereof.

30. A method of **inhibiting** inflammation comprising the step of administering a therapeutically effective amount of an antibody specific for a BAFF-ligand receptor or an epitope thereof.

31. A method of regulating hematopoietic cell development comprising the step of administering a therapeutically effective amount of a BAFF-ligand or an active fragment thereof.

32. A method of treating, **suppressing** or altering an immune response involving a signaling pathway between a BAFF-ligand and its receptor comprising the step of administering an effective amount of an agent capable of interfering with the association between the BAFF-ligand and its receptor.

33. A method of treating hypertension in an animal comprising the step of administering a therapeutically effective amount of a B-cell growth **inhibitor**.

34. The method according to claim 33, wherein the B-cell growth **inhibitor** is selected from the group consisting of: (e) (a) a anti-BAFF ligand molecule or an active fragment thereof; (f) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (g) an antibody specific for BAFF ligand or an active fragment thereof; and (h) an antibody specific for BAFF ligand receptor or an

epitope thereof.

35. The method according to claim 34, wherein the anti-BAFF ligand is soluble.

36. The method according to claim 35, wherein the soluble anti-BAFF ligand is a recombinant anti-BAFF ligand.

37. The method according to claim 34, wherein the anti-BAFF antibody is a monoclonal antibody.

38. The method according to claim 34, wherein the anti-BAFF receptor antibody is a monoclonal antibody.

39. The method according to claim 34, wherein the animal is of mammalian origin.

40. The method according to claim 39, wherein the mammal is human.

41. A method of treating hypertension in an animal comprising the step of administering a therapeutically effective amount of a co-**inhibitor** of B-cell growth and immunoglobulin secretion.

42. A method of treating cardiovascular disorders in an animal comprising the step of administering a therapeutically effective amount of a B-cell growth **inhibitor**.

43. A method of treating cardiovascular disorders in an animal comprising the step of administering a therapeutically effective amount of a co-**inhibitor** of B-cell growth and immunoglobulin production.

44. A method of treating renal disorders in an animal comprising the step of administering a therapeutically effective amount of a B-cell growth **inhibitor**.

45. A method of treating renal disorders in an animal comprising the step of administering a therapeutically effective amount of a co-**inhibitor** of B-cell growth and immunoglobulin production

46. A method of treating B-cell lympho-proliferate disorders comprising the step of administering a therapeutically effective amount of a B-cell growth **inhibitor**.

47. A method of stimulating B-cell production in the treatment of immunosuppressive diseases comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (e) a BAFF ligand or an active fragment thereof; (f) a BAFF ligand or an active fragment thereof and an anti-T antibody; (g) a BAFF ligand or an active fragment thereof and a CD40 ligand; (h) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule; (i) a anti-BAFF ligand molecule or an active fragment thereof; (j) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (k) an antibody specific for BAFF ligand or an active fragment thereof; and (l) an antibody specific for BAFF ligand receptor or an epitope thereof.

48. A method of stimulating B-cell production in the treatment of an immunosuppressive disease comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (i) a BAFF ligand or an active fragment thereof; (j) a BAFF ligand or an active fragment thereof and an anti-T antibody; (k) a BAFF ligand or an active fragment thereof and a CD40 ligand; (l) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule; (m) a anti-BAFF ligand molecule or an active fragment thereof; (n) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (o) an antibody specific for BAFF ligand or an

active fragment thereof; and

49. A method according to claim 48 wherein the immunosuppressive disease is **HIV**.

50. A method according to claim 49 wherein the immunosuppressive disease is associated with an organ transplantation.

51. A method for treating or reducing the advancement, severity or effects of Sjogren's syndrome in a patient comprising the step of administering a pharmaceutical composition comprising a therapeutically effective amount of a BAFF blocking agent and a pharmaceutically acceptable carrier.

52. The method of claim 51 wherein the BAFF blocking agent is selected from the group consisting of a soluble BAFF receptor molecule, an antibody directed against BAFF-ligand and an antibody directed against a BAFF receptor.

53. The method of claim 52 wherein the soluble BAFF receptor further comprises a human immunoglobulin Fc domain.

54. The method of claim 53 wherein the BAFF receptor is TACI.

55. The method of claim 53 wherein the BAFF receptor is BCMA.

56. The method of claim 53 wherein the BAFF receptor is BAFF R.

57. The method of claim 52 wherein the antibody directed against BAFF-ligand is a monoclonal antibody.

58. The method of claim 52 wherein the antibody directed against a BAFF receptor is a monoclonal antibody.

59. The method of claim 58 wherein the monoclonal antibody is directed against TACI.

60. The method of claim 58 wherein the monoclonal antibody is directed against BCMA.

61. The method of claim 58 wherein the monoclonal antibody is directed against BAFF R.

L14 ANSWER 9 OF 27 USPATFULL on STN
2003:133479 METHODS OF TREATING AN UNDESIRED IMMUNE RESPONSE BY BLOCKING THE BINDING OF CD40 LIGAND TO CD40.
ARMITAGE, RICHARD J., BAINBRIDGE ISLAND, WA, UNITED STATES
FANSLOW, WILLIAM C., FEDERAL WAY, WA, UNITED STATES
SPRIGGS, MELANIE K., SEATTLE, WA, UNITED STATES
SRINIVASAN, SUBHASHINI, KIRKLAND, WA, UNITED STATES
GIBSON, MARYLOU G., CARLSBAD, CA, UNITED STATES
US 2003091564 A1 20030515
APPLICATION: US 1999-365940 A1 19990802 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB There is disclosed a polypeptide (CD40-L) and DNA sequences, vectors and transformed host cells useful in providing CD40-L polypeptides. More particularly, this invention provides isolated human and murine CD40-L polypeptides that bind to the extracellular binding region of a CD40 receptor.
CLM What is claimed is:
1. A isolated CD40-L polypeptide selected from the group consisting of: (a) a peptide comprising amino acids 1 through 261, 35 through 261, 34 through 225,113 through 261, 113 through 225, 120 through 261, or 120 through 225 of SEQ ID NO:11 and their complementary strands; and (b)

peptides encoded by DNA which hybridizes to a DNA that encodes a peptide of (a), under stringent conditions (hybridization in 6×SSC at 63° C. overnight; washing in 3×SSC at 55° C.), and which bind to CD40.

2. The isolated CD40-L polypeptide according to claim 1 which is a soluble CD40-L polypeptide.

3. The isolated CD40-L polypeptide according to claim 2 wherein the cysteine at residue 194 of SEQ ID NO:12 is substituted with an amino acid other than cysteine.

4. The isolated CD40-L polypeptide according to claim 3 wherein the substituted amino acid is selected from the group consisting of tryptophan, serine, aspartic acid, and lysine.

5. The isolated CD40-L polypeptide according to claim 2 which further comprises an oligomerizing zipper peptide.

6. The isolated CD40-L polypeptide according to claim 3 which further comprises an oligomerizing zipper peptide.

7. The isolated CD40-L polypeptide according to claim 4 which further comprises an oligomerizing zipper peptide.

8. The isolated DNA according to claim 5 wherein the oligomerzing zipper peptide is a peptide having an amino acid sequence represented by SEQ ID NO:17.

9. The isolated CD40-L polypeptide according to claim 6 wherein the oligomerizing zipper peptide is a peptide having an amino acid sequence represented by SEQ ID NO:17.

10. The isolated CD40-L polypeptide according to claim 7 wherein the oligomerizing zipper peptide is a peptide having an amino acid sequence represented by SEQ ID NO:17.

11. An isolated CD40-L polypeptide which has an amino acid sequence represented by SEQ ID NO:21.

12. The isolated CD40-L polypeptide according to claim 11 wherein the cysteine at residue 180 of SEQ ID NO:21 is substituted with an amino acid other than cysteine.

13. The isolated CD40-L polypeptide according to claim 12 wherein the substituted amino acid is selected from the group consisting of tryptophan, serine, aspartic acid, and lysine.

14. A pharmaceutical composition comprising a CD40-L polypeptide according to claim 2, and a suitable diluent or carrier.

15. A pharmaceutical composition comprising a CD40-L polypeptide according to claim 5, and a suitable diluent or carrier.

16. A pharmaceutical composition comprising a CD40-L polypeptide according to claim 8, and a suitable diluent or carrier.

17. A pharmaceutical composition comprising a CD40-L polypeptide according to claim 9, and a suitable diluent or carrier.

18. A pharmaceutical composition comprising a CD40-L polypeptide according to claim 10, and a suitable diluent or carrier.

19. A pharmaceutical composition comprising a CD40-L polypeptide according to claim 11, and a suitable diluent or carrier.

20. A method of **inhibiting** an undesirable immune response comprising administering an effective amount of a CD40 antagonist selected from the group consisting of a soluble CD40 protein, a CD40 fusion protein, a soluble monomeric CD40-L polypeptide, an antagonistic antibody to CD40, an antagonistic antibody to CD40-L, and combinations thereof.

21. The method according to claim 20, wherein the undesirable immune response is selected from the group consisting of allergy, systemic lupus erythematosis, rheumatoid arthritis, insulin dependent diabetes mellitus, and graft versus host disease.

22. An antibody immunoreactive with CD40-L or a CD40-L immunogen.

23. An antibody according to claim 22 which is a monoclonal antibody.

L14 ANSWER 10 OF 27 USPATFULL on STN
2003:86997 Ligand for herpes simplex virus entry mediator and methods of use.
Ware, Carl, Solana Beach, CA, UNITED STATES
US 2003060605 A1 20030327
APPLICATION: US 2001-967604 A1 20010928 (9)
PRIORITY: US 1997-51964P 19970707 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A novel polypeptide ligand, p30, or LIGHT, for herpes virus entry mediator, HVEM, is provided. LIGHT is useful for modulating immune responses and in **inhibiting** infection and/or subsequent proliferation by herpesvirus. HVEM fusion proteins are also provided. Methods for treating subjects with lymphoid cell disorders, tumors, autoimmune diseases, inflammatory disorders or those having or suspected of having a herpesvirus infection, utilizing p30 and the fusion proteins of the invention, are also provided.

CLM What is claimed is:

1. An isolated or recombinant homotrimeric p30 polypeptide comprising a monomer polypeptide having an apparent molecular weight of about 30 kDa, wherein the homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions.

2. The isolated or recombinant homotrimeric p30 polypeptide of claim 1, wherein the monomer polypeptide comprises isomers having a pI from about 7 to about 8.5.

3. A soluble isolated or recombinant homotrimeric p30 polypeptide lacking a transmembrane domain, wherein the soluble homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions.

4. A liposome comprising a p30 polypeptide, wherein the p30 polypeptide comprises a homotrimeric p30 polypeptide comprising a monomer polypeptide having an apparent molecular weight of about 30 kDa, wherein the homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, or, a soluble homotrimeric p30 polypeptide lacking a transmembrane domain, wherein the soluble homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions.

5. A fusion protein comprising a p30 polypeptide, wherein the p30 polypeptide comprises a homotrimeric p30 polypeptide comprising a monomer polypeptide having an apparent molecular weight of about 30 kDa, wherein the homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR)

polypeptide under physiologic conditions, or, a soluble homotrimeric p30 polypeptide lacking a transmembrane domain, wherein the soluble homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions.

6. The fusion protein of claim 5, wherein the heterologous sequence is a tag.

7. A pharmaceutical composition comprising a p30 polypeptide, wherein the p30 polypeptide comprises a homotrimeric p30 polypeptide comprising a monomer polypeptide having an apparent molecular weight of about 30 kDa, wherein the homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, or, a soluble homotrimeric p30 polypeptide lacking a transmembrane domain, wherein the soluble homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, and a pharmaceutically acceptable excipient.

8. A kit comprising a pharmaceutical composition and printed matter, wherein the pharmaceutical composition comprises a p30 polypeptide, wherein the p30 polypeptide comprises a homotrimeric p30 polypeptide comprising a monomer polypeptide having an apparent molecular weight of about 30 kDa, wherein the homotrimeric polyp eptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, or, a soluble homotrimeric p30 polypeptide lacking a transmembrane domain, wherein the soluble homotrimeric polyp eptide binds to a herpes virus entry mediator (HVEM) polyp eptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, and a pharmaceutically acceptable excipient, wherein the printed matter comprises instructions for a use of the pharmaceutical composition, wherein a use comprises **inhibiting** virus entry into a cell or virus proliferation in a cell.

9. The kit of claim 8, wherein the instructions include use of the pharmaceutical composition for **inhibiting** virus proliferation in a cell or virus entry into a cell in vivo.

10. The kit of claim 8, wherein the virus is a herpesvirus.

11. The kit of claim 10, wherein the virus is a herpes simplex virus (HSV), a cytomegalovirus (CMV), a γ-herpesvirus or an Epstein Barr virus (EBV).

12. The kit of claim 11, wherein the **inhibition** of virus entry in the cell or virus proliferation in a cell is in a mammal.

13. The kit of claim 12, wherein the mammal is a human.

14. A kit comprising a pharmaceutical composition and printed matter, wherein the pharmaceutical composition comprises a p30 polypeptide, wherein the p30 polypeptide comprises a homotrimeric p30 polypeptide comprising a monomer polypeptide having an apparent molecular weight of about 30 kDa, wherein the homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, or, a soluble homotrimeric p30 polypeptide lacking a transmembrane domain, wherein the soluble homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, and a pharmaceutically acceptable excipient, wherein the printed matter comprises instructions for a use of the pharmaceutical composition, wherein a use comprises modulating diseases with unwanted lymphocyte proliferation.

15. The kit of claim 14, wherein the instructions comprise use of the pharmaceutical composition to modulate a T or a B lymphoma or leukemia, or an autoimmune disease.

16. The kit of claim 15, wherein the autoimmune disease is rheumatoid arthritis, insulin-dependent diabetes mellitus, multiple sclerosis, systemic lupus erythematosus or myasthenia gravis.

17. A pharmaceutical composition comprising an expression vector encoding a p30 polypeptide having an apparent molecular weight of about 30 kDa or a p30 polypeptide lacking a transmembrane domain, wherein the p30 polypeptide forms a homotrimeric polypeptide that binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions.

18. A kit comprising a pharmaceutical composition and printed matter, wherein the pharmaceutical composition comprises an expression vector encoding a p30 polypeptide having an apparent molecular weight of about 30 kDa or a p30 polypeptide lacking a transmembrane domain, wherein the p30 polypeptide forms a homotrimeric polypeptide that binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, and a pharmaceutically acceptable excipient, and wherein the printed matter comprises instructions for a use of the pharmaceutical composition, wherein a use comprises targeting of tumor cells or activated lymphocytes.

19. The kit of claim 18, wherein the use comprises treatment of a tumor by direct injection of the pharmaceutical composition into the tumor.

20. A method for inducing a proliferation-inducing signal to a lymphocyte comprising (a) providing a composition that binds to cell surface expressed HVEM, and (b) contacting the lymphocyte with a proliferation-inducing amount of the composition.

21. The method of claim 20, wherein the composition is an anti-HVEM antibody.

22. The method of claim 20, wherein providing the composition comprises providing a p30 polypeptide, a soluble p30 polypeptide, a liposome-associated p30 polypeptide, or a vector encoding a p30 polypeptide or a cell expressing a recombinant p30 as a cell-associated p30 polypeptide.

23. The method of claim 20, wherein the lymphocyte is a T cell.

24. The method of claim 20, wherein the lymphocyte is a B cell.

25. The method of claim 20, wherein the lymphocyte is contacted in vivo.

26. A method for **inhibiting** a p30 polypeptide-mediated cellular response comprising (a) providing an composition that **inhibits** binding of a cell surface expressed p30 polypeptide to a cell surface expressed HVEM or LTβR, and (b) contacting the cell expressing the cell surface expressed p30 polypeptide or the cell surface expressed HVEM or LTβR with an amount of the composition sufficient to **inhibit** a p30 polypeptide-mediated cellular response.

27. The method of claim 26, wherein the cell is contacted with the composition in vivo.

28. The method of claim 26, wherein the **inhibited** p30 polypeptide-mediated cellular response comprises **inhibition** of a lymphocyte cellular response.

29. The method of claim 28, wherein the **inhibited** lymphocyte response is lymphocyte proliferation.

30. The method of claim 28, wherein the **inhibited** lymphocyte is a pathogenic effector cell.

31. The method of claim 28, wherein the **inhibited** lymphocyte response modulates a T or a B lymphoma or leukemia or an autoimmune disease.

32. The method of claim 31, wherein the autoimmune disease is rheumatoid arthritis, insulin-dependent diabetes mellitus, multiple sclerosis, systemic lupus erythematosus or myasthenia gravis.

33. The method of claim 28, wherein the **inhibited** lymphocyte response modulates a reaction to a transplant.

34. The method of claim 26, wherein the contacted cell expresses HVEM and the composition is a soluble p30 polypeptide.

35. The method of claim 26, wherein the contacted cell expresses LTβR and the composition is a soluble p30 polypeptide.

36. The method of claim 26, wherein the contacted cell expresses p30 polypeptide on its cell surface and the composition is a soluble HVEM polypeptide.

37. The method of claim 26, wherein the contacted cell expresses p30 polypeptide on its cell surface and the composition is an anti-p30 an tibody .

38. A method for treating tumors comprising (a) providing a pharmaceutical composition comprising an expression vector encoding a p30 polypeptide having an apparent molecular weight of about 30 kDa or a p30 polypeptide lacking a transmembrane domain, wherein the p30 polypeptide forms a homotrimeric polyp eptide that binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, and (b) directly injecting the pharmaceutical composition into the tumor.

39. A method of modulating a lymphotoxin beta receptor (LTβR)-mediated cellular response, the method comprising: (a) providing a composition that **inhibits** binding of an LTβR to a p30 polypeptide; and (b) contacting a cell expressing the LTβR or the p30 polypeptide with an amount of the composition sufficient to modulate the lymphotoxin β receptor (LTβR)-mediated cellular response.

40. The method of claim 39, wherein the cell expresses LTβR and the composition comprises a pharmaceutical composition wherein the pharmaceutical composition comprises a p30 polypeptide comprising a homotrimeric p30 polypeptide comprising a monomer polypeptide having an apparent molecular weight of about 30 kDa, wherein the homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, or, a soluble homotrimeric p30 polypeptide lacking a transmembrane domain, wherein the soluble homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, and a pharmaceutically acceptable excipient.

41. The method of claim 39, wherein the cell expresses a p30 polypeptide and the composition comprises an anti-p30 antibody.

42. The method of claim 39, wherein the lymphotoxin β receptor

(LTβR)-mediated cellular response comprises binding of a herpesvirus to a cell.

43. The method of claim 42, wherein herpesvirus is blocked from entry into the cell.

44. The method of claim 42, wherein the herpesvirus is a herpes simplex virus (HSV), a cytomegalovirus (CMV), a γ-herpesvirus or an Epstein Barr virus (EBV).

45. A method for **inhibiting** virus production in a cell, the method comprising (a) providing a p30 polypeptide; and, (b) contacting a cell infected with a herpesvirus or a cell susceptible to infection by a herpesvirus with an effective amount of a p30 polypeptide, thereby **inhibiting** herpesvirus production in the cell.

46. The method of claim 45, wherein the entry of the herpesvirus into the cell is **inhibited**.

47. The method of claim 45, wherein the contacting is in vivo and the p30 composition is provided as a pharmaceutical composition, wherein the pharmaceutical composition comprises a homotrimeric p30 polypeptide comprising a monomer polypeptide having an apparent molecular weight of about 30 kDa, wherein the homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, or, a soluble homotrimeric p30 polypeptide lacking a transmembrane domain, wherein the soluble homotrimeric polypeptide binds to a herpes virus entry mediator (HVEM) polypeptide or a lymphotoxin β receptor (LTβR) polypeptide under physiologic conditions, and a pharmaceutically acceptable excipient.

48. The method of claim 45, wherein the virus is a herpes simplex virus (HSV), a cytomegalovirus (CMV), a γ-herpesvirus or an Epstein Barr virus (EBV).

49. The method of claim 45, wherein the contacting is in a mammal.

50. The method of claim 49, wherein the mammal is a human.

51. A method for treating a subject having or at risk of having a herpesvirus infection or a disorder associated with herpesvirus infection comprising contacting the subject with an amount of a LTβR or TNFR1 agonist sufficient to treat herpesvirus infection.

52. The method of claim 51, wherein the herpesvirus comprises, CMV, a β-herpesvirusor a γ-herpesvirus.

53. The method of claim 51, wherein the agonist comprises a ligand or an antibody.

54. The method of claim 53, wherein the ligand comprises a polypeptide.

55. The method of claim 54, wherein the polypeptide comprises p30 (LIGHT), LTα, TNF or LTα1β2.

56. The method of claim 53, wherein the antibody is fully human.

57. The method of claim 53, wherein the antibody is humanized.

58. The method of claim 51, wherein virus proliferation is reduced in the subject following treatment.

59. The method of claim 51, wherein virus nucleic acid replication or protein expression is reduced in the subject following treatment.

60. The method of claim 51, wherein the subject is at risk of virus reactivation from latency.

61. The method of claim 51, wherein the subject is or is at risk of being immunosuppressed.

62. The method of claim 51, wherein the subject has **HIV** infection or a tumor.

63. The method of claim 51, wherein the subject has or is at risk of having a blood or bone marrow, organ, or tissue transplant.

64. The method of claim 51, wherein the subject is a neonate.

65. The method of claim 51, wherein the agonist does not produce substantial apoptosis in cells infected with the virus.

66. The method of claim 51, wherein the disorder comprises pneumonia, arteriosclerosis, CMV hepatitis, CMV retinitis, CMV pneumonitis, CMV nephritis or CMV mononucleosis.

67. The method of claim 51, wherein the disorder comprises child febrile illness, cytomegalaic inclusion disease or a demyelinating disease.

68. The method of claim 67, wherein the demyelinating disease comprises multiple sclerosis.

69. The method of claim 51, wherein the disorder comprises Kaposis sarcoma, Hodgkins leukemia or non-Hodgkins leukemia or lymphoma.

70. The method of claim 51, further comprising contacting the subject with an antiviral agent.

71. A method for identifying a compound that **inhibits** CMV infection, comprising: a) contacting LTβR or TNFR1 with a test compound under conditions allowing binding; b) measuring LTβR or TNFR1 activity in the presence of the test compound; and c) comparing activity in the presence of the test compound to the absence of the test compound, wherein an increase in LTβR or TNFR1 activity in the presence of the test compound identifies the test compound as a compound that **inhibits** CMV infection.

72. The method of claim 71, wherein the test compound is a library of compounds.

73. The method of claim 72, wherein the library of compounds is a peptide or small molecule library.

74. The method of claim 71, wherein the activity comprises activation of an NFkB target gene.

75. The method of claim 74, wherein the NFkB target gene comprises ICAM1, VCAM1, interleukin-8 (IL-8) or secondary lymphoid organ chemokine (SLC).

76. The method of claim 74, wherein the activity comprises activation of IFNgamma or IFNbeta gene expression.

77. The method of claim 71, further comprising testing the identified compound for **inhibiting** CMV infection in cells.

78. The method of claim 71, further comprising testing the identified compound for **inhibiting** CMV infection of animals.

79. A method for identifying a compound that **inhibits** CMV infection,

comprising: a) contacting a cell that expresses LTβR or TNFR1 in the presence of CMV with a test compound under conditions allowing binding between LTβR or TNFR1 and the test compound; b) measuring CMV proliferation, replication, protein expression or cytopathicity in the presence of the test compound; and c) comparing CMV proliferation, replication, protein expression or cytopathicity in the presence of the test compound to the absence of the test compound, wherein a decrease in CMV proliferation, replication, protein expression or cytopathicity in the presence of the test compound identifies the test compound as a compound that **inhibits** CMV infection.

80. The method of claim 79, wherein the test compound is a library of compounds.

81. A pharmaceutical composition comprising a LTβR or TNFR1 agonist and an antiviral agent in a pharmaceutical carrier.

82. The pharmaceutical composition of claim 81, wherein the LTβR or TNFR1 agonist comprises a ligand or an antibody.

83. The pharmaceutical composition of claim 82, wherein the ligand comprises a polypeptide.

84. The pharmaceutical composition of claim 83, wherein the polypeptide comprises p30 (LIGHT), LTα, TNF or LTα1β2.

85. The pharmaceutical composition of claim 82, wherein the antibody is fully human.

86. The pharmaceutical composition of claim 82, wherein the antibody is humanized.

87. A pharmaceutical composition comprising an antibody having a LTβR or TNFR1 agonist activity.

88. The pharmaceutical composition of claim 87, wherein the antibody is a monoclonal antibody denoted 3C8, 3H4 or 4H8, or an antigen binding fragment thereof.

89. An antibody having the binding specificity of monoclonal antibody denoted 3C8, 3H4 or 4H8.

90. The antibody of claim 89, wherein the antibody is humanized.

91. The antibody of claim 89, wherein the antibody is human.

92. A pharmaceutical composition comprising the antibody of claim 89.

93. A kit comprising an LTβR or TNFR1 agonist, and instructions for use in treating a subject having or at risk of having a herpesvirus infection.

94. A kit comprising a pharmaceutical composition comprising an LTβR or TNFR1 agonist and instructions for use in treating a subject having or at risk of having a herpesvirus infection.

95. The kit of claims 93 or 94, further comprising an antiviral agent.

L14 ANSWER 11 OF 27 USPATFULL on STN
2003:64296 NUCLEIC ACIDS ENCODING CD100 MOLECULES.
HALL, KATHRYN T., BOSTON, MA, UNITED STATES
FREEMAN, GORDON J., BROOKLINE, MA, UNITED STATES
SCHULTZE, JOACHIM L., BROOKLINE, MA, UNITED STATES
BOUSSIOTIS, VASSILIKI A., BROOKLINE, MA, UNITED STATES

NADLER, LEE M., NEWTON, MA, UNITED STATES
DANA-FARBER CANCER INSTITUTE (U.S. corporation)
US 2003044416 A1 20030306
APPLICATION: US 1995-556422 A1 19951109 (8)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Isolated nucleic acid molecules encoding novel CD100 molecules which stimulate a leukocyte response, such as a B cell response, including B cell aggregation, B cell differentiation, B cell survival, and/or T cell proliferationare disclosed. These novel molecules have a certain homology to semaphorins, proteins which are growth cone guidance molecules that are critical for guiding growing axons of neurons to their targets. In addition to isolated nucleic acids molecules, antisense nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced are also described. The invention further provides isolated CD100 proteins, fusion proteins and active fragments thereof. Diagnostic and therapeutic methods utilizing compositions of the invention are also provided.
CLM What is claimed is:

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a CD100 antigen.

2. The isolated nucleic acid molecule of claim 1, which is a cDNA.

3. The isolated nucleic acid molecule of claim 2, wherein the CD100 antigen is human.

4. The nucleic acid of claim 3 comprising a nucleotide sequence shown in FIG. 1, SEQ ID NO: 1.

5. The isolated nucleic acid of claim 4 comprising the coding region.

6. The isolated nucleic acid of claim 3 derived from a hematopoietic cell which hybridizes under high stringency conditions to a nucleic acid molecule comprising a nucleotide sequence shown in FIG. 1, SEQ ID NO: 1.

7. An isolated nucleic molecule comprising a nucleotide sequence encoding a protein wherein the protein comprises an amino acid sequence having at least about 80% overall amino acid sequence identity with an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

8. The isolated nucleic acid molecule of claim 7, wherein the protein has a CD100 activity.

9. An isolated nucleic acid molecule encoding an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

10. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein, wherein the protein comprises a semaphorin domain having an amino acid sequence at least 80% identical to an amino acid sequence in FIG. 2, SEQ ID NO: 2.

11. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein, wherein the protein comprises a) a semaphorin domain having an amino acid sequence at least 60% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2; and b) an immunoglobulin-like domain having an amino acid sequence at least 50% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

12. The isolated nucleic acid molecule of claim 11, further comprising c) a cytoplasmic domain having an amino acid sequence at least 50% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

13. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a protein, wherein the protein comprises an extracellular

domain having an amino acid sequence at least 80% identical to a amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

14. An isolated nucleic acid molecule encoding a CD100 fusion protein comprising a nucleotide sequence encoding a first peptide having an amino acid sequence at least 80% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2 and a nucleic sequence encoding a second peptide corresponding to a moiety that alters the solubility, binding affinity or valency of the first peptide.

15. The isolated nucleic acid molecule of claim 14, wherein the first peptide comprises an extracellular domain of a human CD100 antigen.

16. The isolated nucleic acid molecule of claim 14, wherein the first peptide comprises a semaphorin domain of human CD100 antigen.

17. The isolated nucleic acid molecule of claim 14, wherein the second peptide comprises an immunoglobulin constant region.

18. An isolated nucleic acid molecule comprising a nucleotide sequence encoding a peptide comprising a fragment of at least about 30 amino acids of the sequence shown in FIG. 2, SEQ ID NO: 2.

19. The isolated nucleic acid molecule of claim 18, wherein the peptide has a CD100 activity.

20. An isolated nucleic acid molecule of claim 1, which is antisense to the nucleic acid molecule of claim 1.

21. An isolated nucleic acid molecule of claim 1, which is antisense to the nucleic acid molecule of claim 4.

31. An isolated nucleic acid molecule of claim 1, which is antisense to the nucleic acid molecule of claim 5.

32. A vector comprising a nucleotide sequence encoding a CD100 antigen.

33. A vector comprising a nucleotide sequence encoding a protein comprising an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

34. A host cell comprising the vector of claim 32.

35. A host cell comprising the vector of claim 33.

36. A method for producing a CD100 antigen comprising culturing a host cell of claim 34 in a suitable medium such that the CD100 antigen is produced.

37. A method for producing a CD100 antigen comprising culturing a host cell of claim 35 in a suitable medium such that the CD100 antigen is produced.

38. An isolated protein having a CD100 activity.

39. The isolated protein of claim 38, which is human.

40. The isolated protein of claim 39, wherein the protein comprises an amino acid sequence having at least about 80% overall amino acid sequence identity with an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

41. The isolated protein of claim 40, wherein the protein comprises an amino acid sequence having at least about 90% overall amino acid sequence identity with an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

42. The isolated protein of claim 40, wherein the protein comprises an amino acid sequence having at least about 95% overall amino acid sequence identity with an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

43. The isolated protein of claim 39, wherein the protein CD100 activity.

44. The isolated protein of claim 43, which comprises an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

45. An isolated protein comprising a semaphorin domain having an amino acid sequence at least 80% identical to an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

46. The isolated protein of claim 45 having an amino acid sequence at least 90% identical to an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

47. An isolated protein comprising a) a semaphorin domain having an amino acid sequence at least 60% identical to an amino acid sequence shown in FIG. 2 SEQ ID NO: 2); and b) an immunoglobulin-like domain having an amino acid sequence that is at least 50% identical to an amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

48. The isolated protein of claim 47, further comprising c) a cytoplasmic domain having an amino acid sequence at least 50% identical to amino acid sequence shown in FIG. 2 (SEQ ID NO: 2).

49. An isolated protein comprising an extracellular domain having an amino acid sequence at least 80% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2.

50. An CD100 fusion protein comprising a first peptide having an amino acid sequence at least 80% identical to an amino acid sequence shown in FIG. 2, SEQ ID NO: 2 and a second peptide corresponding to a moiety that alters the solubility, binding affinity or valency of the first peptide.

51. The fusion protein of claim 50, wherein the first peptide comprises an extracellular domain of a human CD100 antigen.

52. The fusion protein of claim 50, wherein the first peptide comprises a semaphorin domain of a human CD100 antigen.

53. The fusion protein of claim 50, wherein the second peptide comprises an immunoglobulin constant region.

54. A peptide comprising a fragment of at least about 30 amino acids of the sequence shown in FIG. 2, SEQ ID NO: 2.

55. The peptide of claim 54, which has a CD100 activity.

56. The peptide of claim 54, wherein the fragment is at least about 40 amino acids in length.

57. The peptide of claim 56 which has a CD100 activity.

58. The peptide of claim 56, wherein the fragment is at least about 50 amino acid in length.

59. The peptide of claim 58, which has a CD100 activity.

60. A pharmaceutical composition comprising the protein of claim 44 and a pharmaceutically acceptable carrier.

61. A pharmaceutical composition comprising the protein of claim 50 and

a pharmaceutically acceptable carrier.

62. A pharmaceutical composition comprising the protein of claim 55 and a pharmaceutically acceptable carrier.

63. A vaccine composition comprising at least one antigen and a first agent which stimulates a CD100 ligand-associated signal.

64. The composition of claim 63, further comprising a second agent which stimulates a CD40-associated signal.

65. A method for modulating a B cell response comprising contacting the B cell with an agent which modulates a CD100 ligand-associated signal in the B cell, such that a response by the B cell is modulated.

66. The method of claim 65, wherein the agent stimulates a CD100 ligand associated signal, such that a response by the B cell is stimulated.

67. The method of claim 66, wherein the agent is a stimulatory form of a CD100 antigen.

68. The method of claim 67, wherein the stimulatory form of a CD100 antigen is a soluble form of a CD100 antigen.

69. The method of claim 68, wherein the soluble form of a CD100 antigen is an immunoglobulin fusion protein.

70. The method of claim 67, wherein the stimulatory form of a CD100 antigen is attached to a solid phase support.

71. The method of claim 68, wherein the solid phase support is a cell membrane.

72. The method of claim 66, further comprising contacting the B cell with a second agent which provides a stimulatory signal to the B cell.

73. The method of claim 72, wherein the second agent stimulates a CD40 associated signal in the B cell.

74. The method of claim 73, wherein the second agent is a CD40 ligand.

75. The method of claim 66, further comprising contacting the B cells with T cells.

76. The method of claim 75, further comprising contacting the B cells with at least one antigen.

77. The method of claim 65, wherein the agent **inhibits** a CD100 ligand-associated signal, such that a response by the B cell is **inhibited**.

78. The method of claim 77, wherein the agent interacts with CD100.

79. The method of claim 78, wherein the agent is an antibody to CD100.

80. The method of claim 77, wherein the agent interacts with a CD100 ligand.

81. The method of claim 80, wherein the agent is an **inhibitory** form of CD100.

82. The method of claim 65, wherein the B cell response is B cell aggregation.

83. The method of claim 65, wherein the B cell response is B cell differentiation.

84. The method of claim 83, wherein the B cells differentiate into plasma cells.

85. The method of claim 83, wherein the B cells differentiate to memory B cells.

86. The method of claim 65, wherein the B cell response is B cell viability.

87. The method of claim 65, wherein contacting the cells is in a subject, such that a response by the B cell is modulated in the subject.

88. The method of claim 87, wherein the agent stimulates a B cell response in a subject infected with a pathogen, such that elimination of the pathogen by the subject is enhanced.

89. The method of claim 87, wherein the agent stimulates a B cell response in a subject having a low grade lymphoma, such that the B cell differentiation in the subject is stimulated.

90. The method of claim 88, further comprising administering to the subject a second agent which stimulates a CD40-associated signal in the B cell.

91. The method of claim 87, wherein the agent **inhibits** a B cell response in an allergic subject, such that the B cell response by the subject to an allergen is **inhibited**.

92. The method of claim 87, wherein the agent **inhibits** a B cell response in a subject having a large cell lymphoma, such that differentiation of the B cell in the subject is **inhibited**.

93. A method for modulating an interaction between an immune cell and a nerve cell in a subject, comprising administering to a subject an agent which modulates a CD100 ligand-associated signal in the nerve cell, such that modulation of the interaction between the immune cell and the nerve cell in the subject occurs.

94. A method for modulating axonal growth of a neuron, comprising contacting the neuron with a modulating form of CD100, such that axonal growth is modulated.

95. A method for modulating a T cell response, comprising contacting the T cell with an agent which modulates a CD100 ligand-induced signal in the T cell, such that a response in the T cell is modulated.

96. The method of claim 95, wherein a T cell is contacted with an agent which stimulates a CD100 ligand-induced signal in the T cell, such that a response in the T cell is stimulated.

97. The method of claim 96, further comprising contacting the T cell with an agent which provides a primary activation signal to the T cell.

98. The method of claim 95, wherein the T cell is contacted with an agent which **inhibits** a CD100 ligand-induced signal in the T cell, such that a response in the T cell is **inhibited**.

L14 ANSWER 12 OF 27 USPATFULL on STN
2003:30210 Methods of producing a library and methods of selecting polynucleotides of interest.
Zauderer, Maurice, Pittsford, NY, UNITED STATES
Smith, Ernest S., Ontario, NY, UNITED STATES
University of Rochester (U.S. corporation)
US 2003022157 A1 20030130

APPLICATION: US 2001-818991 A1 20010328 (9)
PRIORITY: US 2000-192586P 20000328 (60)
US 2000-203343P 20000510 (60)
US 2001-263226P 20010123 (60)
US 2001-271426P 20010227 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a high efficiency method of introducing DNA into linear DNA viruses such as poxvirus, a method of producing libraries in linear DNA viruses such as poxvirus, and methods of selecting polynucleotides of interest based on cell nonviability or other phenotypes.
CLM What is claimed is:

1. A method of selecting a target polynucleotide, comprising: (a) introducing into a population of host cells a library of insert polynucleotides; wherein at least one of said insert polynucleotides comprises the target polynucleotide; and wherein expression of said target polynucleotide directly or indirectly promotes host cell death; (b) culturing said host cells; and (c) collecting insert polynucleotides from those host cells which undergo cell death.

2. The method of claim 1, further comprising: (d) introducing said collected polynucleotides into a population of host cells, wherein expression of said target polynucleotide directly or indirectly promotes host cell death; (e) culturing said host cells; and (f) collecting insert polynucleotides from those host cells which undergo cell death.

3. The method of claim 2, further comprising repeating steps (d)-(f) one or more times, thereby enriching for said target polynucleotide.

4. The method claim 3, further comprising purifying said collected polynucleotides.

5. The method of claim 1, wherein said cell death is the result of a cellular effect selected from the group consisting of cell lysis, expression of a suicide gene product, a cytotoxic T-lymphocyte-induced lytic event, apoptosis, loss of viability, loss of membrane integrity, loss of structural stability, cell disruption, disruption of cytoskeletal elements, inability to maintain membrane potential, arrest of cell cycle, inability to generate energy, growth arrest, cytotoxic effects, cytostatic effects, genotoxic effects, and growth **suppressive** effects.

6. The method of claim 1, wherein said population of host cells is selected from the group consisting of: tumor cells, metastatic tumor cells, primary cells, transformed primary cells, immortalized primary cells, dividing cells, non dividing cells, terminally differentiated cells, pluripotent stem cells, committed progenitor cells, uncommitted stem cells, progenitor cells, muscle cells, epithelial cells, nervous system cells, circulatory system cells, respiratory system cells, endocrine cells, endocrine-associated cells, skeletal system cells, connective tissue cells, musculoskeletal cells, chondrocytes, osteoblasts, osteoclasts, myocytes, fully differentiated blood cells, fully differentiated epidermal cells, neurons, glial cells, kidney cells, liver cells, muscle cell progenitors, epithelial cell progenitors, nervous system cell progenitors, circulatory system cell progenitors, respiratory system cell progenitors, endocrine cell progenitors, endocrine-associated cell progenitors, skeletal system cell progenitors, connective tissue cell progenitors, musculoskeletal cell progenitors, chondrocyte progenitors, osteoblast progenitors, osteoclast progenitors, myocyte progenitors, blood cell progenitors, epidermal cell progenitors, neuron progenitors, glial cell progenitors, kidney cell progenitors, liver cell progenitors and any combination thereof.

7. The method of claim 1, wherein said host cells are adherent to a solid support.

8. The method of claim 7, wherein said solid support is selected from the group consisting of: tissue culture plastic, glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, magnetite, soluble material, partially soluble material, insoluble material, magnetic material, and nonmagnetic material.

9. The method of claim 7, wherein said solid support has a structure selected from the group consisting of: spherical, bead-like, bead, cylindrical, test tube-like, tube-like, tube, rod-like, rod, flat, sheet-like, sheet, test strip, strip-like, strip, bead, microbead, well, plate, tissue culture plate, petri plate, microplate, microtiter plate, flask, stick, vial, and paddle.

10. The method of claim 1, wherein said library of insert polynucleotides is selected from the group consisting of: a cDNA library, a genomic library, a combinatorial polynucleotide library, a library of natural polynucleotides, a library of artificial polynucleotides, a library of polynucleotides endogenous to said host cells, a library of polynucleotides exogenous to said host cells, an antisense library, and any combination thereof.

11. The method of claim 1, wherein expression of said target polynucleotide directly or indirectly promotes cell death upon exposure of said host cells to an agent.

12. The method of claim 11, wherein expression of said target polynucleotide indirectly promotes cell death upon exposure of said host cells to an agent.

13. The method of claim 11, wherein expression of said target polynucleotide directly promotes cell death upon exposure of said host cells to an agent.

14. The method of claim 11, wherein said agent is a member selected from the group consisting of: a physical agent, a chemical agent, and a biological agent.

15. The method of claim 11, wherein said physical agent is selected from the group consisting of: radiation, UV radiation, gamma radiation, infrared radiation, visible light, increased temperature, and decreased temperature.

16. The method of claim 11, wherein said chemical agent is selected from the group consisting of: a chemotherapeutic agent, a cytotoxic agent, and a DNA damaging agent.

17. The method of claim 11, wherein said biological agent is selected from the group consisting of: an antisense construct, an infectious agent, a therapeutic agent, an antibody, a cytotoxic T-lymphocyte (CTL), a ligand, a hapten, an epitope, and a receptor.

18. The method of claim 11, wherein said biological agent is selected from the group consisting of: an infectious agent, a therapeutic agent, an antibody, a ligand, a hapten, an epitope, and a receptor; and wherein said biological agent is conjugated to a toxin.

19. The method of claim 11, wherein said biological agent effects cell death by a process selected from the group consisting of: CTL-induced cytotoxicity, antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity.

20. The method of claim 11, wherein said biological agent comprises a cytotoxic T lymphocyte (CTL), wherein said CTL expresses surface CD4, wherein said target polynucleotide encodes a polypeptide, and wherein

said polypeptide is processed and presented in association with a class II major histocompatibility molecule (MHC).

21. The method of claim 1, wherein expression of said target polynucleotide effects a cellular process selected from the group consisting of cellular differentiation, growth regulation, cellular proliferation, apoptosis, and hormonal response.

22. The method of claim 5, wherein said cell death is the result of apoptosis.

23. The method of claim 22, wherein apoptosis is induced through expression of a apoptosis-related gene product which directly promotes apoptosis.

24. The method of claim 22, wherein apoptosis is induced through expression of an apoptosis-related gene product which indirectly promotes apoptosis.

25. The method of claim 24, wherein said apoptosis-related gene product comprises a death domain containing receptor expressed on the surface of said host cells, and wherein said host cells are contacted with a ligand for said death domain containing receptor.

26. The method of claim 22, wherein said host cells are adherent to a solid support.

27. The method of claim 26, wherein those cells which have undergone apoptosis are released from said support.

28. The method of claim 27, wherein said released host cells, or contents thereof, are collected by removing the liquid medium in which said host cells are cultured.

29. The method of claim 26, wherein those host cells which have undergone apoptosis are fully or partially lysed, thereby releasing their cytoplasmic contents into the liquid medium in which said host cells are cultured.

30. The method of claim 29, wherein said released host cell contents are collected by removing the liquid medium in which said host cells are cultured.

31. The method of claim 5, wherein said cell death is the result of a cytotoxic T-lymphocyte-induced lytic event.

32. The method of claim 31, wherein said target polynucleotide encodes a target epitope for a cytotoxic T lymphocyte (CTL).

33. The method of claim 32, wherein said CTL is a CD4+ CTL.

34. The method of claim 32, wherein said target epitope is expressed on the surface of said host cells in the context of a native MHC molecule expressed on said host cell, and wherein said host cells are contacted with CTLs which are restricted for said MHC molecule and specific for said target epitope.

35. The method of claim 34, wherein said MHC molecule is selected from the group consisting of a class I MHC molecule and a class II MHC molecule.

36. The method of claim 35, wherein said MHC molecule is a class II MHC molecule.

37. The method of claim 36, wherein said target polynucleotide is fused to a polynucleotide encoding Ii-80 fragment of the class II MHC molecule

invariant chain.

38. The method of claim 31, wherein said host cells are adherent to a solid support.

39. The method of claim 38, wherein those cells which have undergone a CTL-mediated lytic event are released from said support.

40. The method of claim 39, wherein said released host cells, or contents thereof, are collected by removing the liquid medium in which said host cells are cultured.

41. The method of claim 38, wherein those host cells which have undergone a CTL-mediated lytic event are fully or partially lysed, thereby releasing their cytoplasmic contents into the liquid medium in which said host cells are cultured.

42. The method of claim 41, wherein said released host cell contents are collected by removing the liquid medium in which said host cells are cultured.

43. The method of claim 5, wherein said cell death is the result of expression of a suicide gene product.

44. The method of claim 43, wherein said suicide gene product is selected from the group consisting of a diphtheria toxin A chain polypeptide, a Pseudomonas exotoxin A chain polypeptide, a ricin A chain polypeptide, an abrin A chain polypeptide, a modeccin A chain polypeptide, and an alpha-sarcin polypeptide.

45. The method of claim 43, wherein said host cells are progenitor cells comprising a suicide gene operably associated with a tissue-restricted promoter; wherein expression of said target polynucleotide directly or indirectly induces transcription of said tissue-restricted promoter, resulting in expression of said suicide gene; and wherein expression of said suicide gene promotes death of those progenitor cells comprising said target polynucleotide.

46. The method of claim 45, wherein said host cell is a RAW cell, and wherein said suicide gene is operably associated with the TRAP promoter.

47. The method of claim 46, wherein said target polynucleotide directly or indirectly regulates osteoclast differentiation.

48. The method of claim 46, wherein said suicide gene encodes the Diphtheria toxin A subunit.

49. The method of claim 45, wherein said tissue-restricted promoter is identified by gene expression profiling of said host cells under different physical conditions in microarrays of ordered cDNA libraries.

50. The method of claim 49, wherein said expression profiling compares gene expression under different physical conditions in host cells infected with a eukaryotic virus expression vector, wherein said eukaryotic virus expression vector is the vector used to construct said library of polynucleotides.

51. The method of claim 43, wherein said host cells are non-dividing cells comprising a suicide gene operably associated with a proliferation-specific promoter; wherein expression of said target polynucleotide directly or indirectly induces transcription of said proliferation-specific promoter, resulting in expression of said suicide gene; and wherein expression of said suicide gene promotes death of those non-dividing host cells comprising said target polynucleotide.

52. The method of claim 51, wherein said proliferation-specific promoter

is identified by gene expression profiling of said host cells under different physical conditions in microarrays of ordered cDNA libraries.

53. The method of claim 52, wherein said express ion pro filing compares gene expression under different physical conditions in host cells infected with a eukaryotic virus expression vector, wherein said eukaryotic virus expression vector is the vector used to construct said library of polynucleotides.

54. The method of claim 43, wherein said host cells are adherent to a solid support.

55. The method of claim 54, wherein those host cells expressing said suicide gene product are released from said support.

56. The method of claim 55, wherein said released host cells, or contents thereof, are collected by removing the liquid medium in which said host cells are cultured.

57. The method of claim 54, wherein those host cells expressing said suicide gene product are fully or partially lysed, thereby releasing their cytoplasmic contents into the liquid medium in which said host cells are cultured.

58. The method of claim 57, wherein said released host cell contents are collected by removing the liquid medium in which said host cells are cultured.

59. The method claim 5, wherein cell death occurs within a period selected from the group consisting of: 48 hours after expression of said insert polynucleotide, 24 hours after expression of said insert polynucleotide, and 12 hours after expression of said insert polynucleotide.

60. The method of claim 1, wherein said library of polynucleotides is constructed in a eukaryotic virus vector.

61. The method of claim 60, wherein said host cells are infected with said library at an MOI selected from the group consisting of: from about 1 to about 10, about 1 to about 5, and about 1.

62. The method of claim 60, wherein said eukaryotic virus vector is an animal virus vector.

63. The method of claim 60, wherein said eukaryotic virus vector is a plant virus vector.

64. The method of any one of claims 60, wherein said eukaryotic virus vector is capable of producing infectious viral particles in cells selected from the group consisting of insect cells, plant cells, and mammalian cells.

65. The method of claim 64, wherein said eukaryotic virus vector is attenuated.

66. The method of claim 64, wherein said eukaryotic virus vector is capable of producing infectious viral particles in mammalian cells.

67. The method of claim 65, wherein said attenuation is by genetic mutation.

68. The method of claim 65, wherein said attenuation is by reversible **inhibition** of virus replication.

69. The method of claim 60, wherein the naturally-occurring genome of said eukaryotic virus vector is DNA.

70. The method of claim 69, wherein the naturally-occurring genome of said eukaryotic virus vector is linear, double-stranded DNA.

71. The method of claim 70, wherein said eukaryotic virus vector is selected from the group consisting of an adenovirus vector, a herpesvirus vector and a poxvirus vector.

72. The method of claim 71, wherein said eukaryotic virus vector is a poxvirus vector.

73. The method of claim 72, wherein said poxvirus vector is selected from the group consisting of an orthopoxvirus vector, an avipoxvirus vector, a capripoxvirus vector, a leporipoxvirus vector, and a suipoxvirus vector.

74. The method of claim 73, wherein said poxvirus vector is an orthopoxvirus vector selected from the group consisting of a vaccinia virus vector and a raccoon poxvirus vector.

75. The method of claim 74, wherein said orthopoxvirus vector is a vaccinia virus vector.

76. The method of claim 74, wherein said host cells are permissive for the production of infectious viral particles of said virus.

77. The method of claim 75, wherein said vaccinia virus is attenuated.

78. The method of claim 77, wherein said attenuation is by genetic mutation.

79. The method of claim 77, wherein said attenuation is by reversible **inhibition** of virus replication.

80. The method of claim 77, wherein said vaccinia virus vector is derived from strain MVA.

81. The method of claim 77, wherein said vaccinia virus vector is derived from strain D4R.

82. The method of claim 72, wherein said insert polynucleotide is in operably associated with a transcriptional control sequence.

83. The method of claim 82, wherein said transcriptional control sequence functions in the cytoplasm of a poxvirus-infected cell.

84. The method of claim 82, wherein said transcriptional control sequence comprises a promoter.

85. The method of claim 84, wherein said promoter is constitutive.

86. The method of claim 85, wherein said promoter is a vaccinia virus p7.5 promoter.

87. The method of claim 85, wherein said promoter is a synthetic early/late promoter.

88. The method of claim 82, wherein said transcriptional control sequence comprises a transcriptional termination region.

89. The method of claim 60, wherein said library of insert polynucleotides is constructed in said eukaryotic virus vector by a method comprising: (a) providing host cells comprising a linear DNA virus genome which has been cleaved to produce a first viral fragment and a second viral fragment, wherein said first fragment is nonhomologous with said second fragment; (b) providing a population of

transfer plasmids comprising said insert polynucleotides in operable association with a vector transcriptional control region, a 5' flanking region, and a 3' flanking region; wherein said 5' flanking region is homologous to said first viral fragment and said 3' flanking region is homologous to said second viral fragment; and wherein said transfer plasmids are capable of homologous recombination with said first and second viral fragments such that a viable virus genome is formed; (c) introducing said transfer plasmids into said host cells under conditions wherein a transfer plasmid and said first and second viral fragments undergo in vivo homologous recombination, thereby producing a viable modified virus genome comprising an insert polynucleotide; and (d) collecting said modified virus genome.

90. The method of claim 89, wherein said first and second viral fragments are produced by infecting said host cells with a virus comprising said linear DNA virus genome, and wherein said virus genome is cleaved in vivo.

91. The method of claim 89, wherein said first and second viral fragments are produced by cleaving an isolated linear DNA virus genome in vitro, and wherein said first and second viral fragments are introduced into said host cells.

92. The method of claim 91, wherein said virus genome comprises a first recognition site for a first restriction endonuclease; and wherein said first and second viral fragments are produced by digesting said viral genome with said first restriction endonuclease, and isolating said first and second viral fragments.

93. The method of claim 92, wherein said virus genome further comprises a second recognition site for a second restriction endonuclease; and wherein said first and second viral fragments are produced by digesting said viral genome with said first restriction endonuclease and said second restriction endonuclease, and isolating said first and second viral fragments.

94. The method of claim 93, wherein said first and second recognition sites are physically arranged in said genome such that the region extending between said first and second viral fragments is not essential for virus infectivity.

95. The method of claim 89, wherein said modified virus genome is packaged in an infectious viral particle.

96. The method of claim 89, wherein said modified virus genome is defective in an essential gene and said host cell comprises a complementing copy of said essential gene.

97. The method of claim 96, wherein said complementing copy of said essential gene is operably associated with an inducible promoter.

98. The method of claim 97, wherein said inducible promoter is selected from the group consisting of: a differentiation-induced promoter, a cell type-restricted promoter, a tissue-restricted promoter, a temporally-regulated promoter, a spatially-regulated promoter, a proliferation-induced promoter, a cell-cycle specific promoter.

99. The method of claim 89, wherein said linear DNA virus genome is a herpes virus genome.

100. The method of claim 89, wherein said linear DNA virus genome is an adenovirus genome.

101. The method of claim 89, wherein said linear DNA virus genome is a poxvirus genome.

102. The method of claim 101, wherein said poxvirus genome is a vaccinia virus genome.

103. The method of claim 96, wherein said linear DNA virus genome is a pox virus genome, and wherein said essential gene encodes uracil DNA glycosylase.

104. The method of claim 101, wherein said host cell further comprises a helper virus, and wherein said host cell is non-permissive for the production of infectious virus particles of said helper virus.

105. The method of claim 104, wherein said helper virus is an avipoxvirus.

106. The method of claim 105, wherein said helper virus is a fowlpox virus.

107. The method of claim 101, wherein the 5' and 3' flanking regions of said transfer plasmids are capable of homologous recombination with a vaccinia virus thymidine kinase gene.

108. The method of claim 107, wherein the 5' and 3' flanking regions of said transfer plasmids are capable of homologous recombination with a vaccinia virus HindIII J fragment.

109. The method of claim 107, wherein said transfer plasmid comprises an insert polynucleotide operably associated with a promoter selected from the group consisting of a vaccinia virus p7.5 promoter, a synthetic early/late promoter, and a vaccinia virus MH5 early/late promoter.

110. A method of selecting a target polynucleotide, comprising: (a) introducing into a population of host cells a library of insert polynucleotides; wherein said library is constructed in a linear DNA virus vector; wherein at least one of said insert polynucleotides comprises said target polynucleotide; and wherein expression of said target polynucleotide directly or indirectly **inhibits** death of a host cell comprising said target polynucleotide; (b) culturing said host cells; and (c) collecting insert polynucleotides from those host cells which do not undergo cell death.

111. The method of claim 110, further comprising: (d) introducing said collected polynucleotides into a population of host cells, and wherein expression of said target polynucleotide directly or indirectly **inhibits** death of a host cell comprising said target polynucleotide; (e) culturing said host cells; and (f) collecting insert polynucleotides from those host cells which do not undergo cell death.

112. The method of claim 111, further comprising repeating steps (d)-(f) one or more times, thereby enriching for said target polynucleotide.

113. The method of claim 112, further comprising purifying said collected polynucleotides.

114. A method of selecting a target polynucleotide, comprising: (a) introducing into a population of host cells a library of insert polynucleotides; wherein said library is constructed in a linear DNA virus vector; wherein at least one of said insert polynucleotides comprises said target polynucleotide; wherein exposure of said host cells to an agent promotes cell death; and wherein expression of said target polynucleotide directly or indirectly **inhibits** death of a host cell comprising said target polynucleotide; (b) culturing said host cells; (c) exposing said host cells to said agent; and (d) collecting insert polynucleotides from those host cells which do not undergo cell death.

115. The method of claim 114, further comprising: (e) introducing said

collected polynucleotides into a population of host cells, wherein exposure of said host cells to an agent promotes cell death; and wherein expression of said target polynucleotide directly or indirectly **inhibits** death of a host cell comprising said target polynucleotide; (f) culturing said host cells; (g) exposing said host cells to said agent; and (h) collecting insert polynucleotides from those host cells which do not undergo cell death.

116. The method of claim 115, further comprising repeating steps (e)-(h) one or more times, thereby enriching for said target polynucleotide.

117. The method of claim 116, further comprising purifying said collected polynucleotides.

118. A method of selecting a target polynucleotide, comprising: (a) introducing into a population of host cells a library of insert polynucleotides; wherein said library is constructed in a linear DNA virus vector; wherein at least one of said insert polynucleotides comprises the target polynucleotide; and wherein expression of said target polynucleotide directly or indirectly alters a phenotype of a host cell comprising said target polynucleotide; (b) culturing said host cells; and (c) collecting insert polynucleotides from those host cells which exhibit said altered phenotype.

119. The method of claim 118, further comprising: (d) introducing said collected polynucleotides into a population of host cells, and wherein expression of said target polynucleotide directly or indirectly alters a phenotype of a host cell comprising said target polynucleotide; (e) culturing said host cells; and (f) collecting insert polynucleotides from those host cells which exhibit said altered phenotype.

120. The method of claim 119, further comprising repeating steps (d)-(f) one or more times, thereby enriching for said target polynucleotide.

121. The method of claim 120, further comprising purifying said collected polynucleotides.

122. The method of claim 118, wherein said altered phenotype is the expression of a reporter gene product.

123. The method of claim 122, wherein said reporter gene product is selected from the group consisting of an epitope, chloramphenicol acetyl transferase (CAT), green fluorescent protein (GFP), blue fluorescent protein (BFP), yellow fluorescent protein (YFP), red fluorescent protein (RFP), luciferase and β-galactosidase.

124. The method of claim 122, wherein expression of said target polynucleotide indirectly promotes expression of said reporter gene product in said host cells upon exposure of said host cells to an agent.

125. The method of claim 124, wherein said agent is selected from the group consisting of: an infectious agent, a therapeutic agent, an antibody, a ligand, a hapten, an epitope, and a receptor; and wherein said agent is labeled.

126. The method of claim 124, wherein said target polynucleotide encodes a secreted product.

127. A method of selecting a target polynucleotide encoding a secreted product, comprising: (a) dividing host cells comprising a library of insert polynucleotides into pools; wherein said library is constructed in a linear DNA virus vector; wherein at least one of said insert polynucleotides comprises the target polynucleotide; and wherein expression of said target polynucleotide and secretion of said secreted product directly or indirectly alters a phenotype of an indicator cell; (b) culturing said host cell pools in the presence of indicator cells;

(c) collecting insert polynucleotides from those host cell pools in which said indicator cells exhibit an altered phenotype.

128. The method of claim 127, further comprising: (d) introducing said collected polynucleotides into host cells; (e) dividing the host cells of (d) into pools; wherein expression of said target polynucleotide directly or indirectly alters a phenotype of said indicator cells; (f) culturing said host cell pools in the presence of indicator cells; (g) collecting insert polynucleotides from those host cell pools in which said indicator cells exhibit an altered phenotype.

129. The method of claim 128, further comprising repeating steps (d)-(g) one or more times, thereby enriching for said target polynucleotide.

130. The method claim 129, further comprising purifying said collected polynucleotides.

131. The method of claim 127, wherein said altered phenotype is the expression of a reporter gene product.

132. The method of claim 131, wherein said reporter gene product is selected from the group consisting of an epitope, chloramphenicol acetyl transferase (CAT), green fluorescent protein (GFP), blue fluorescent protein (BFP), yellow fluorescent protein (YFP), red fluorescent protein (RFP), luciferase and β-galactosidase.

133. The method of claim 127, wherein said target polynucleotide alters a phenotype of said indicator cells upon exposure of said host cells to an agent.

134. The method of claim 127, wherein expression of said target polynucleotide effects a cellular process selected from the group consisting of cellular differentiation, growth regulation, cellular proliferation, apoptosis, and hormonal response.

135. The method of claim 127, wherein said indicator cells are progenitor cells comprising a selectable gene product operably associated with a tissue-restricted promoter; wherein expression of said target polynucleotide directly or indirectly induces transcription of said tissue-restricted promoter, resulting in expression of said selectable gene product.

136. The method of claim 135, wherein said indicator cell is a RAW cell, and wherein the marker gene is operably associated with the TRAP promoter.

137. The method of claim 136, wherein said target polynucleotide directly or indirectly regulates osteoclast differentiation in said indicator cells.

L14 ANSWER 13 OF 27 USPATFULL on STN
2003:23307 Generation of antigen specific T **suppressor** cells for treatment of rejection.
Suciu-Foca, Nicole, New York, NY, UNITED STATES
Cortesini, Raffaello, New York, NY, UNITED STATES
Liu, Zhuoro, New York, NY, UNITED STATES
Chang, Chih-Chao, New York, NY, UNITED STATES
US 2003017143 A1 20030123
APPLICATION: US 2000-746311 A1 20001221 (9)
DOCUMENT TYPE: Utility; APPLICATION.

AB This invention provides a method of generating antigen specific allospecific human **suppressor** **CD8**+CD28- T cells. This invention also provides a method of generating xenospecific human **suppressor** **CD8**+CD28- T cells. This invention further provides a method of generating allopeptide antigen specific human **suppressor** **CD8**+CD28- T

cells. Methods of treatment for reduction of risk of rejection of allografts and xenografts and autoimmune diseases using the human **suppressor** CD8+CD28- T cells so produced are also provides, as are methods of preventing rejection and autoimmune diseases, and vaccines comprising the produced **suppressor** T cells. Methods of diagnosis to determine whether a level of immuno-**suppressant** therapy requires a reduction are provided.

CLM What is claimed is:

1. A method of generating antigen specific allospecific human **suppressor** CD8+CD28- T cells which comprises: a) obtaining peripheral blood T cells from a subject; b) stimulating by multiple priming a T cell line from the T cells obtained in step (a) with allogeneic antigen presenting cells (APCs), said APCs expressing an MHC class I antigen recognized by the primed T cell line and an MHC class II antigen recognized by CD4+ T helper cells from said primed T cell line; c) isolating primed **CD8+ T cells** and CD4+ T helper cells from the T cell line stimulated in step (b); d) isolating primed **CD8**+CD28- T cells from the isolated primed **CD8+ T cells** of step (c); e) detecting **suppression** by the primed **CD8**+CD28- T cells isolated in step (d) of interaction between the CD4+ T helper cells isolated in step (c) and allogeneic antigen presenting cells (APCs) expressing the same MHC class I antigen and the same MHC class II antigen expressed by the APCs used to stimulate the T cell line of step (b), thereby identifying antigen specific allospecific human **suppressor** CD8+CD28- T cells; and f) expanding in culture the antigen specific allospecific human **suppressor** CD8+CD28- T cells identified in step (e), thereby generating the antigen specific allospecific human **suppressor** CD8+CD28- T cells.

2. The method of claim 1 wherein the MHC class I antigen is an HLA-A or HLA-B antigen expressed by the APC used for priming in step (b).

3. The method of claim 1 wherein the MHC class II antigen is an HLA-DR, HLA-DQ or HLA-DP antigen.

4. Antigen specific allospecific human **suppressor** CD8+ CD28+ T cells produced by the method of claim 1.

5. A method of generating xenospecific human **suppressor** CD8+CD28- T cells which comprises: a) obtaining peripheral blood T cells from a human subject; b) stimulating by multiple priming a human T cell line from the T cells obtained in step (a) with a xenogeneic antigen presenting cells (APCs), said APCs expressing a xenogeneic MHC class I antigen and a xenogeneic MHC class II antigen; c) isolating primed human **CD8+ T cells** and human CD4+ T helper cells from the T cell line stimulated in step (b); d) isolating primed human **CD8**+CD28- T cells from the isolated primed human **CD8+ T cells** of step (c); e) detecting **suppression** by the primed human **CD8**+CD28- T cells isolated in step (d) of interaction between the human CD4+ T helper cells isolated in step (c) and xenogeneic antigen presenting cells (APCs) expressing the same xenogeneic MHC class I antigen and xenogeneic MHC class II antigen expressed by the xenogeneic APCs used to stimulate the human T cell line of step (b), thereby identifying xenospecific human **suppressor** CD8+CD28- T cells; and f) expanding the xenospecific human **suppressor** CD8+CD28- T cells identified in step (e), thereby generating the xenospecific human **suppressor** CD8+CD28- T cells.

6. The method of claim 5 wherein the xenospecific mammalian antigen presenting cells (APCs) are selected from pig or primate APCs.

7. The method of claim 5 wherein the xenogeneic MHC class I antigen is selected from the group consisting of swine histocompatibility leukocyte antigen (SLA) class-I and MHC class II antigen is selected from the group consisting of swine histocompatibility leukocyte antigen (SLA) class-II.

8. Xenospecific human **suppressor CD8+** CD28+ T cells produced by the method of claim 5.

9. A method of generating allopeptide antigen specific human **suppressor CD8+**CD28- T cells which comprises: a) obtaining peripheral blood T cells from a subject; b) stimulating by multiple priming a T cell line from the T cells obtained in step (a) with autologous antigen presenting cells (APCs) pulsed with an allopeptide, said allopeptide comprising an amino acid sequence comprising both MHC class I and MHC class II amino acid sequences wherein the amino acid sequences are binding sequences and are recognized by the primed T cell line; c) isolating primed **CD8+ T cells** and CD4+ T helper cells from the T cell line stimulated in step (b); d) isolating primed **CD8+**CD28- T cells from the isolated primed **CD8+ T cells** of step (c); e) detecting **suppression** by the primed **CD8+**CD28- T cells isolated in step (d) of interaction between the CD4+ T helper cells isolated in step (c) and autologous antigen presenting cells (APCs) expressing the same MHC class I and MHC class II binding motifs as expressed by the APCs used to stimulate the T cell line of step (b), thereby identifying allopeptide antigen specific human **suppressor CD8+**CD28- T cells; and f) expanding the allopeptide antigen specific human **suppressor CD8+**CD28- T cells identified in step (e), thereby generating the allopeptide antigen specific human **suppressor CD8+**CD28- T cells.

10. The method of claim 9 wherein the allopeptide is selected from the group consisting of a peptide antigen, a whole protein antigen, tat-DR4 peptide or a peptide comprising an amino acid sequence of a hypervariable region of HLA-DR B1.

11. Antigen specific human **suppressor CD8+**CD28- T cells produced by the method of claim 9.

12. A method of determining whether a level of immunosuppresant therapy given to a subject undergoing the level immunosuppression therapy requires a reduction which comprises: a) obtaining a blood sample from the subject; and b) determining the presence of T **suppressor** cells present in the sample, the presence of T **suppressor** cells indicating that the subject requires the reduction of immunosuppresant therapy.

13. The method of claim 12 wherein the T **suppressor** cells are **suppressor CD8+**CD28- T cells.

14. A method of reducing the risk of rejection of an allograft in a subject undergoing immunosuppression therapy which comprises: a) obtaining a blood sample from the subject; b) removing T **suppressor** cells from the blood sample; c) expanding the T **suppressor** cells of step (b); and d) reintroducing the expanded T **suppressor** cells of step (b) into the subject.

15. The method of claim 14 wherein the T **suppressor** cells are **suppressor CD8+**CD28- T cells.

16. A method of reducing the level of rejection of an allograft in a subject undergoing immunosuppression therapy which comprises administering to the subject the T **suppressor** cells produced by the method of claim 1, thereby preventing rejection of the tissue or organ transplant in the subject.

17. A method of reducing the level of rejection of an allograft in a subject undergoing immunosuppression therapy which comprises administering to the subject the T **suppressor** cells produced by the method of claim 9, thereby preventing rejection of the tissue or organ transplant in the subject.

18. A method of preventing rejection of an allograft in a subject which

comprises: a) obtaining a blood sample from the subject; b) removing T **suppressor** cells from the blood sample; c) expanding the T **suppressor** cells of step (b); and d) reintroducing the expanded T **suppressor** cells of step (b) into the subject, thereby preventing the rejection of the allograft in the subject.

18. A method of preventing rejection of an allograft in a subject which comprises administering the T **suppressor** cells produced by the method of claim 1 to the subject, thereby preventing rejection of the allograft in the subject.

19. A method of preventing rejection of an allograft in a subject which comprises administering the T **suppressor** cells produced by the method of claim 9 to the subject, thereby preventing rejection of the allograft in the subject.

20. A method of preventing rejection of a xenograft in a subject which comprises: a) obtaining a blood sample from the subject; b) removing T **suppressor** cells from the blood sample; c) expanding the T **suppressor** cells of step (b); and d) reintroducing the expanded T **suppressor** cells of step (b) into the subject, thereby preventing the rejection of the xenograft in the subject.

21. The method of claim 20 wherein the T **suppressor** cells are **suppressor CD8**+CD28- T cells.

22. A method of preventing rejection of a xenograft in a subject which comprises administering the T **suppressor** cells produced by the method of claim 5 to the subject, thereby preventing rejection of the xenograft in the subject.

23. A method of preventing autoimmune disease in a subject which comprises: a) obtaining a blood sample from the subject; b) removing T **suppressor** cells from the blood sample; c) expanding the T **suppressor** cells of step (b); and d) reintroducing the expanded T **suppressor** cells of step (b) into the subject, thereby preventing autoimmune disease in the subject.

24. The method of claim 23 wherein the T **suppressor** cells are **suppressor CD8**+CD28- T cells.

25. A method of preventing autoimmune disease in a subject which comprises administering the T **suppressor** cells produced by the method of claim 1 to the subject, thereby preventing autoimmune disease in the subject.

26. A method of preventing autoimmune disease in a subject which comprises administering the T **suppressor** cells produced by the method of claim 9 to the subject, thereby preventing autoimmune disease in the subject.

27. A vaccine comprising allospecific T **suppressor** cells stimulated by APCs expressing an MHC class I antigen and an MHC class II antigen which T **suppressor** cells **suppress** an interaction between CD4+ T helper cells and allogeneic antigen presenting cells (APCs) expressing the same MHC class I antigen and the same MHC class II antigen expressed by the APCs used to stimulate the allospecific T **suppressor** cells.

28. The vaccine of claim 27 wherein the APCs are allogeneic APCs said APCs expressing an MHC class I antigen recognized by the T **suppressor** cells and an MHC class II antigen recognized by allogeneic CD4+ T helper cells.

29. The vaccine of claim 27 wherein the APCs are APCs pulsed with an allopeptide, said allopeptide comprising an amino acid sequence having both MHC class I and MHC class II binding motifs wherein both motifs are

recognized by the stimulated T **suppressor** cells.

30. The vaccine of claim 27 wherein the T **suppressor** cells are **suppressor CD8**+CD28- T cells.

31. A vaccine comprising xenospecific T **suppressor** cells stimulated by APCs expressing a xenospecific MHC class I antigen and a xenogeneic MHC class II antigen which xenogeneic T **suppressor** cells **suppress** an interaction between CD4+ T helper cells and xenogeneic antigen presenting cells (APCs) expressing the same xenogeneic MHC class I antigen and xenogeneic MHC class II antigen expressed by the APCs used to stimulate the xenospecific T **suppressor** cells.

32. The vaccine of claim 31 wherein the T **suppressor** cells are **suppressor CD8**+CD28- T cells.

33. A method of inducing anergic T helper cells which comprises: a) incubating antigen presenting cells (APC) with allospecific T **suppressor** cells (Ts); b) overexpressing in the APC mRNA which encodes at least one monocyte **inhibitory** receptor (MIR), in a mixture of cells comprising the APCs from step (a), wherein overexpression of MIR transmits negative **inhibitory** signals to recruit an **inhibitory** signaling molecule, thyrosine phosphatase SHP-1 such that the APC are rendered tolerogenic; and c) incubating the APCs from step (b) with T helper cells (Th) to induce Th anergy.

34. The method of claim 33, wherein the monocyte **inhibitory** receptor (MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

35. The method of claim 33, wherein the Ts are allospecific human **suppressor CD8**+CD28- T cells.

36. The method of claim 33, wherein the Ts are xenospecific human **suppressor CD8**+CD28- T cells.

37. The method of claim 33, wherein the Ts allopeptide are antigen specific human **suppressor CD8**+CD28- T cells.

38. A method of generating a tolerogenic antigen presenting cell (APC) which comprises: a) contacting the APC with Ts; and b) overexpressing mRNA which encodes an MIR in the APC, thereby generating a tolerogenic antigen presenting cell (APC).

39. The method of claim 38, wherein the monocyte **inhibitory** receptor (MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

40. The method of claim 38, wherein the Ts are antigen specific allospecific human **suppressor CD8**+CD28- T cells.

41. The method of claim 38, wherein the Ts are xenospecific human **suppressor CD8**+CD28- T cells.

42. The method of claim 38, wherein the Ts are allopeptide antigen specific human **suppressor CD8**+CD28- T cells.

43. A method of reducing the level of rejection of an allograft tissue or organ in a subject who is a transplant recipient of the allograft tissue or organ which comprises administering to the subject tolerogenic antigen presenting cells (APC) which overexpress monocyte **inhibitory** receptor (MIR) wherein the APC have been incubated with Ts prior to overexpression of MIR, thereby inducing Th anergy so as to prevent rejection of the tissue or organ allograft in the subject.

44. The method of claim 43, wherein the monocyte **inhibitory** receptor

(MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

45. The method of claim 43, wherein the Ts are allospecific human **suppressor** CD8+CD28- T cells.

46. The method of claim 43, wherein the Ts are xenospecific human **suppressor** CD8+CD28- T cells.

47. The method of claim 43, wherein the Ts are allopeptide antigen specific human **suppressor** CD8+CD28- T cells.

48. A method of **suppressing** an autoimmune disease in a subject which comprises: a) contacting antigen presenting cells (APC) of the subject with T **suppressor** cells (Ts) specific for the antigen which induces the autoimmune disease; and b) administering to the subject the APC of step (a), thereby inducing tolerance to the antigen so as to **suppress** the autoimmune disease in the subject.

49. The method of claim 48, wherein the monocyte **inhibitory** receptor (MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

50. The method of claim 48, wherein the Ts are allospecific human **suppressor** CD8+CD28- T cells.

51. The method of claim 48, wherein the Ts are xenospecific human **suppressor** CD8+CD28- T cells.

52. The method of claim 48, wherein the Ts are allopeptide antigen specific human **suppressor** CD8+CD28- T cells.

53. A method of **suppressing** an autoimmune disease in a subject which comprises: a) overexpressing monocyte **inhibitory** receptor (MIR) in antigen presenting cells (APC) of the subject, which APC present the antigen which induces the autoimmune disease and are genetically engineered to overexpress MIR; and b) administering to the subject the APC of step (a), thereby inducing tolerance to the antigen so as to **suppress** the autoimmune disease in the subject.

54. The method of claim 53, wherein the monocyte **inhibitory** receptor (MIR) is selected from the group consisting of ILT4 (MIR-10), ILT2 (MIR7), and ILT3.

55. A method of inducing tolerance to an allograft tissue or organ in a subject which comprises administering to the subject tolerogenic antigen presenting cells (APC) which overexpress monocyte **inhibitory** receptor (MIR), thereby inducing tolerance to the allograft in the subject.

56. A method of inducing tolerance to a xenograft tissue or organ in a subject which comprises administering to the subject tolerogenic antigen presenting cells (APC) which overexpress monocyte **inhibitory** receptor (MIR), thereby inducing tolerance to the xenograft in the subject.

57. An antigen presenting cell (APC) which overexpresses ILT3, wherein the APC comprises a retroviral vector comprising a nucleic acid sequence which encodes ILT3 and overexpresses ILT3.

58. The APC of claim 57, wherein the APC is an APC from a subject who is a tissue or organ transplant donor.

59. A method of inducing tolerance to a xenograft tissue or organ transplant in a subject which comprises: a) introducing to an antigen presenting cell (APC) of a tissue or organ transplant donor a vector which overexpresses ILT3, wherein the vector comprises a nucleic acid sequence which encodes ILT3 and overexpresses ILT3; and b)

administering the APC of step (a) to the subject, thereby inducing tolerance to the xenograft in the subject.

60. The method of claim 59, wherein the vector is a retroviral vector.

61. A method of inducing tolerance to an allograft tissue or organ in a subject which comprises: a) introducing to an antigen presenting cell (APC) of a tissue or organ transplant donor a vector which overexpresses ILT3, wherein the vector comprises a nucleic acid sequence which encodes ILT3 and overexpresses ILT3; and b) administering the APC of step (a) to the subject, thereby inducing tolerance to the allograft in the subject.

62. The method of claim 61, wherein the vector is a retroviral vector.

63. A method of treating an autoimmune disease in a subject which comprises: a) introducing to an antigen presenting cell (APC) of a subject having the autoimmune disease a vector which overexpresses ILT3, wherein the vector comprises a nucleic acid sequence which encodes ILT3 and overexpresses ILT3; and b) administering the APC of step (a) to the subject, thereby treating the autoimmune disease in the subject.

64. The method of claim 63, wherein the vector is a retroviral vector.

65. The method of claim 63, wherein the autoimmune disease is selected from the group consisting of diabetes, rheumatoid arthritis, and multiple sclerosis.

66. A method of determining the appearance of T **suppressor** (Ts) cells which comprises detecting the level of expression of ILT3, ILT4, and ILT2 protein in APCs of a subject, wherein the subject is a xenograft tissue or organ transplant recipient which comprises: a) obtaining a sample from the subject; and b) detecting in the sample of step (a) overexpression of mRNA which encodes the ILT3, ILT4, and ILT2 protein in the APC of the subject, wherein detection of overexpression of mRNA which encodes the ILT3, ILT4, and ILT2 protein indicates the appearance of T **suppressor** cells in the subject.

67. The method of claim 66, wherein the Ts are xenospecific human **suppressor CD8**+CD28- T cells.

68. A method of determining the appearance of T **suppressor** (Ts) cells which comprises detecting the level of expression of ILT3, ILT4, and ILT2 protein in APCs of a subject, wherein the subject is an allograft tissue or organ transplant recipient which comprises: a) obtaining a sample from the subject; and b) detecting in the sample of step (a) overexpression of mRNA which encodes the ILT3, ILT4, and ILT2 protein in the APC of the subject, wherein detection of overexpression of mRNA which encodes the ILT3, ILT4, and ILT2 protein indicates the appearance of T **suppressor** cells in the subject.

69. The method of claim 68, wherein the Ts are allospecific human **suppressor CD8**+CD28- T cells.

L14 ANSWER 14 OF 27 USPATFULL on STN
2002:300810 Peroxiredoxin drugs for treatment of **HIV**-1 infection and methods of use thereof.
Lynn, Ralf Geiben, Watertown, MA, UNITED STATES
Walker, Bruce D., Milton, MA, UNITED STATES
US 2002168353 A1 20021114
APPLICATION: US 2002-57593 A1 20020125 (10)
PRIORITY: US 2001-278234P 20010323 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention includes compositions comprising substantially purified

peroxiredoxin that are useful in methods for the treatment and prevention of **HIV**-1 infection. The invention also includes methods for the treatment and prevention of **HIV**-1 infection comprising contacting a composition of the invention with a human patient. Additionally, the invention includes antibodies and kits useful in the treatment and prevention of **HIV**-1 infection.

CLM What is claimed is:

1. A method of treating **HIV**-1 infection, the method comprising contacting a cell susceptible to **HIV**-1 infection with an amount of peroxiredoxin sufficient to **inhibit** infection of the cell by **HIV**-1.

2. The method of claim 1, wherein the peroxiredoxin is selected from the group consisting of type I peroxiredoxin and type II peroxiredoxin.

3. The method of claim 1, wherein the peroxiredoxin is protease-resistant.

4. A method of decreasing the infectivity of **HIV**-1, if any is present, in a biological sample, the method comprising: (a) identifying a biological sample in which a reduction or elimination of **HIV**-1 infectivity is desirable; and (b) contacting the biological sample with an amount of peroxiredoxin sufficient to decrease the infectivity of **HIV**-1 in the biological sample.

5. The method of claim 3, wherein the biological sample is selected from the group consisting of: blood, plasma, serum, saliva, semen, cervical secretions, saliva, urine, breast milk, cell culture medium, and amniotic fluids.

6. The method of claim 3, wherein the peroxiredoxin is selected from the group consisting of: type I peroxiredoxin and type II peroxiredoxin.

7. The method of claim 3, wherein the peroxiredoxin is protease-resistant.

8. The method of claim 3, wherein the amount of peroxiredoxin is at least about 5 μg/ml of the biological sample volume.

9. The method of claim 3 wherein the amount of peroxiredoxin is at least about 10 μg/ml of the biological sample volume.

10. A method of treating **HIV**-1 infection, the method comprising contacting a cell susceptible to **HIV**-1 infection with an amount of manganese dismutase sufficient to **inhibit** infection of the cell by **HIV**-1.

11. A method of treating **HIV**-1 infection, the method comprising introducing into a cell susceptible to **HIV**-1 infection a DNA molecule encoding a peroxiredoxin, and expressing the peroxiredoxin in an amount sufficient to **inhibit** infection of the cell by the **HIV**-1.

12. A method of treating **HIV**-1 infection in a subject, the method comprising introducing into the subject a cell that expresses a peroxiredoxin in an amount sufficient to **inhibit** infection of an endogenous cell of the subject, the endogenous cell being susceptible to **HIV**-1 infection.

13. A biological sample purification system to reduce the number of **HIV**-1 particles in a biological sample, comprising a peroxiredoxin linked to a surface.

14. A biological sample purification system to reduce the number of **HIV**-1 particles in a biological sample, comprising a peroxiredoxin linked to a surface, wherein contacting said biological sample and said biological sample purification system results in a reduction in the number of **HIV**-1 particles present in the biological sample.

15. The purification system of claim 14, wherein said surface is a bead, chip, column, or matrix.

16. A pharmaceutical composition for the treatment or prevention of **HIV** infection in a subject, comprising a peroxiredoxin and a pharmaceutically acceptable carrier.

17. A kit comprising in one or more containers the pharmaceutical composition of claim 16.

L14 ANSWER 15 OF 27 USPATFULL on STN
2002:297432 Non-stochastic generation of genetic vaccines.
Short, Jay M., Rancho Santa Fe, CA, United States
Diversa Corporation, San Diego, CA, United States (U.S. corporation)
US 6479258 B1 20021112
APPLICATION: US 2000-495052 20000131 (9)
PRIORITY: US 1995-8311P 19951207 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides methods of obtaining vaccines by use of non-stochastic methods of directed evolution (DirectEvolution.TM.). These methods include non-stochastic polynucleotide site-satuaration mutagenesis (Gene Site Saturation Mutagenesis.TM.) and non-stochastic polynucleotide reassembly (GeneReassembly.TM.). Through use of the claimed methods, vectors can be obtained which exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like.

CLM What is claimed is:

1. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising: creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set, wherein optimization is achieved by at least one directed evolution method in any combination, permutation and iterative manner.

2. The method of claim 1, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

3. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization,or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, the method comprising: screening a library Qf non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created.

4. The method of claim 3, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

5. A method for obtaining an immunomodulatory polynucleotide that has an optimized modulatory effect on an immune response as compared to the response prior to optimization, or encodes a polypeptide that has an optimized modulatory effect on an immune response as compared to the

response prior to optimization, the method comprising: a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that has, or encodes a polypeptide that has, a modulatory effect on an immune response induced by a vector; wherein the optimized non-stochastically generated polynucleotide or the polypeptide encoded by the non-stochastically generated polynucleotide exhibits an enhanced ability to modulate an immune response compared to a parental polynucleotide from which the library was created; and whereby optimization is achieved using one or more directed evolution methods in any combination, permutation, and iterative manner.

6. The method of claim 5, wherein said optimized modulatory effect on an immune response is induced by a genetic vaccine vector.

7. The method of any claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide is incorporated into a vector.

8. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide, or a polypeptide encoded by the optimized non-stochastically generated polynucleotide, is administered in conjunction with a vector.

9. The method of any of claims 1, 3 or 5, wherein the library of non-stochastically generated progeny polynucleotides is created by a process selected from gene reassembly or oligonucleotide-directed saturation mutagenesis, and any combination, permutation and iterative manner.

10. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide that has a modulatory effect on an immune response is obtained by: a) non-stochastically reassembling at least two parental template polynucleotide, each of which is, or encodes a molecule that is, involved in modulating an immune response; wherein the first and second parental templates differ from each other in two or more nucleotides, to produce a library of non-stochastically generated polynucleotides; and b) screening the library to identity at least one optimized non-stochastically generated polynucleotide that exhibits, either by itself or through the encoded molecule, an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created.

11. The method of claim 10, wherein the method further comprises the steps of: c) subjecting an optimized non-stochastically generated polynucleotide to a further round of non-stochastic reassembly with at least one additional polynucleotide, which is the same or different from the first and second polynucleotides, to produce a further library of recombinant polynucleotides; d) screening the library produced in c) to identify at least one further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response in comparison to a parental polynucleotide from which the library was created; and e) optionally repeating c) and d) as necessary, until a desirable further optimized non-stochastically generated polynucleotide that exhibits an enhanced ability to modulate an immune response than a form of the nucleic acid from which the library was created.

12. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that interacts with a cellular receptor involved in mediating an immune response; wherein the polypeptide acts as an agonist or antagonist of the receptor.

13. The method of claim 12, wherein the cellular receptor is a

macrophage scavenger receptor.

14. The method of claim 12, wherein the cellular receptor is selected from the group consisting of a cytokine receptor and a chemokine receptor.

15. The method of claim 14, wherein the chemokine receptor is CCR5 or CCR6.

16. The method of claim 12, wherein the polypeptide mimics the activity of a natural ligand for the receptor but does not induce immune reactivity to said natural ligand.

17. The method of claim 12, wherein the library is screened by: i) expressing the non-stochastically generated progeny polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package; ii) contacting the replicable genetic packages with a plurality of cells that display the receptor; and iii) identifying cells that exhibit a modulation of an immune response mediated by the receptor.

18. The method of claim 17, wherein the replicable genetic package is selected from the group consisting of a bacteriophage, a cell, a spore, and a virus.

19. The method of claim 18, wherein the replicable genetic package is an M13 bacteriophage and the protein is encoded by geneIII or geneVIII.

20. The method of claim 12, which method further comprises introducing the optimized non-stochastically generated polynucleotide into a genetic vaccine vector and administering the vector to a mammal, wherein the peptide or polypeptide is expressed and acts as an agonist or antagonist of the receptor.

21. The method of claim 12, which method further comprises producing the polypeptide encoded by the optimized non-stochastically generated polynucleotide and introducing the polypeptide into a mammal in conjunction with a genetic vaccine vector.

22. The method of claim 12, wherein the optimized non-stochastically generated polynucleotide is inserted into an antigen-encoding nucleotide sequence of a genetic vaccine vector.

23. The method of claim 22, wherein the optimized non-stochastically generated polypeptide is introduced into a nucleotide sequence that encodes an M-loop of an HBsAg polypeptide.

24. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide comprises a nucleotide sequence rich in unmethylated CpG.

25. The method of any of claims 1, 3 or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide that **inhibits** an allergic reaction.

26. The method of claim 25, wherein the polypeptide is selected from the group consisting of interferon-α, interferon-γ, IL-10, IL-12, an antagonist of IL-4, an antagonist of IL-5, and an antagonist of IL-13.

27. The method of 1, wherein the optimized recombinant polynucleotide encodes an antagonist of IL-10.

28. The method of claim 27, wherein the antagonist of IL-10 is soluble or defective IL-10 receptor or IL-20/MDA-7.

29. The method of any of claims 1-6, wherein the optimized non-stochastically generated polynucleotide encodes a co-stimulator.

30. The method of claim 29, wherein the co-stimulator is B7-1 (**CD80**) or B7-2 (**CD86**) and the screening step involves selecting variants with altered activity through CD28 or CTLA-4.

31. The method of claim 29, wherein the co-stimulator is CD1, CD40, CD154 (ligand for CD40) or CD150 (SLAM).

32. The method of claim 29, wherein the co-stimulator is a cytokine.

33. The method of claim 32, wherein the cytokine is selected from the group consisting of IL-1, IL-2, IL-3, IL4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF-α, IFN-α, IFN-γ, and IL-20 (MDA-7).

34. The method of 33, wherein the library of non-stochastically generated polynucleotides is screened by testing the ability of cytokines encoded by the non-stochastically generated polynucleotides to activate cells which contain a receptor for the cytokine.

35. The method of claim 34, wherein the cells contain a heterologous nucleic acid that encodes the receptor for the cytokine.

36. The method of 33, wherein the cytokine is interleukin-12 and the screening is performed by: growing mammalian cells which contain the genetic vaccine vector in a culture medium; and detecting whether T cell proliferation or T cell differentiation is induced by contact with the culture medium.

37. The method of 33, wherein the cytokine is interferon-α and the screening is performed by: i) expressing the non-stochastically generated polynucleotides so that the encoded polypeptides are produced as fusions with a protein displayed on the surface of a replicable genetic package; ii) contacting the replicable genetic packages with a plurality of B cells; and iii) identifying phage library members that are capable of **inhibiting** proliferation of the B cells.

38. The method of claim 33, wherein the immune response of interest is differentiation of T cells to T_{H1} cells and the screening is performed by contacting a population of T cells with the cytokines encoded by the members of the library of recombinant polynucleotides and identifying library members that encode a cytokine that induces the T cells to produce IL-2 and interferon-γ.

39. The method of claim 32, wherein the cytokine encoded by the optimized non-stochastically generated polynucleotide exhibits reduced immunogenicity compared to a cytokine encoded by a non-optimized polynucleotide, and the reduced immunogenicity is detected by introducing a cytokine encoded by the non-stochastically generated polynucleotide into a mammal and determining whether an immune response is induced against the cytokine.

40. The method of claim 29, wherein the co-stimulator is B7-1 (**CD80**) or B7-2 (**CD86**) and the cell is tested for ability to costimulate an immune response.

41. The method of any of claims 1, 3, or 5, wherein the optimized recombinant polynucleotide encodes a cytokine antagonist.

42. The method of claim 41, wherein the cytokine antagonist is selected from the group consisting of a soluble cytokine receptor and a transmembrane cytokine receptor having a defective signal sequence.

43. The method of claim 41, wherein the cytokine antagonist is selected

from the group consisting of ΔIL-1 OR and ΔIL-4R.

44. The method of any of claims 1, 3, or 5, wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H1} immune response.

45. The method of any of claims 1, 3, or 5 wherein the optimized non-stochastically generated polynucleotide encodes a polypeptide capable of inducing a predominantly T_{H2} immune response.

46. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is a decrease in an unwanted modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having a decreased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having decreased antigenicity with respect to at least one host recipient of said molecule.

47. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is an increase in a desirable modulatory effect on an immune response; whereby application of the method can. be used to generate a molecule having an increased ability to elicit an immune response from a host recipient of said molecule, where said recipient can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having increased antigenicity with respect to at least one host recipient of said molecule.

48. The method of any of claims 1, 3, or 5, wherein said optimized modulatory effect on an immune response is both a decrease in a first unwanted modulatory effect on an immune response as well as an increase in a second desirable modulatory effect on an immune response; whereby application of the method can be used to generate a molecule having both a decreased ability to elicit a first immune response from a first host recipient of said molecule as well as a an increased ability to elicit a second immune response from a second host recipient of said molecule; whereby the first and the second recipient hosts can be the same or different; whereby each of the first and the second recipient hosts can be a human or an animal host; and whereby application of the method can thus be used to generate a molecule having both a first decreased antigenicity with respect to at least one host recipient of said molecule and a second decreased antigenicity with respect to at least one host recipient of said molecule.

49. The method of claim 48, wherein said first and said second modulatory effect on an immune response are evolved for respectively a first and a second module on the same multimodule vaccine vector; whereby a module is exemplified by the following modules, as well as by a fragment derivative or analog thereof: an antigen coding sequence, a polyadenylation sequence, a sequence coding for a co-stimulatory molecule, a sequence coding for an inducible repressor or transactivator, a eukaryotic origin or replication, a prokaryotic origin of replication, a sequence coding for a prokaryotic marker, and enhancer, a promoter, and operator, and an intron.

50. The method of any of claims 1, 3, or 5, wherein the optimized modulatory effect on an immune response is comprised of an increase in the stability of the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; whereby application of the method can be used to generate a molecule having an increased stability ex vivo, thus, for example, increasing shelf-life and/or ease of storage and/or length of time before expiration of activity upon storage; and whereby application of the method can also be used to generate a molecule having an increased stability in vivo upon administration to a host recipient,

thus, for example, increasing resistance to digestive acids and/or increasing stability in the circulation and/or any other method of elimination or destruction by the host recipient.

51. The method of any of claims 1, 3, or 5, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in a human host recipient as compared with prior to optimization; whereby application of the method can thus be used to generate an optimized genetic vaccine for human recipients.

52. The method of any of claims 1, 3, or 5, wherein the immunomodulatory (IM) polynucleotide or polypeptide encoded thereby; has an optimized modulatory effect on an immune response in an animal host recipient as compared with prior to optimization; whereby application of the method can thus be used to generate an optimized genetic vaccine for animal recipients, including animals that are farmed or raised by man, animals that are not fanned or raised by man, domesticated animals, and non-domesticated animals.

53. A method for obtaining an optimized polynucleotide that encodes an accessory molecule that improves the transport or presentation of antigens by a cell, the method comprising: a) creating a library of non-stochastically generated polynucleotides by subjecting to optimization by non-stochastic directed evolution a parental polynucleotide set in which is encoded all or part of the accessory molecule; and b) screening the library to identify an optimized non-stochastically generated progeny polynucleotide that encodes a recombinant molecule that confers upon a cell an increased or decreased ability to transport or present an antigen on a surface of the cell compared to an accessory molecule encoded by template polynucleotides not subjected to the non-stochastic reassembly; whereby application of the method can thus be used to generate an optimized molecule for human recipients &/or animal recipients, including animals that are farmed or raised by man, animals that are not farmed or raised by man, domesticated animals, and non-domesticated animals; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

54. The method of claim 53, wherein the screening involves: i) introducing the library of non-stochastically generated polynucleotides into a genetic vaccine vector that encodes an antigen to form a library of vectors; introducing the library of vectors into mammalian cells; and ii) identifying mammalian cells that exhibit increased or decreased immunogenicity to the antigen.

55. The method of claim 53, wherein the accessory molecule comprises a proteasome or a TAP polypeptide.

56. The method of claim 53, wherein the accessory molecule comprises a cytotoxic T-cell inducing sequence.

57. The method of claim 56, wherein the cytotoxic T-cell inducing sequence is obtained from a hepatitis B surface antigen.

58. The method of claim 53, wherein the accessory molecule comprises an immunogenic agonist sequence.

59. A method for obtaining an immunomodulatory polynucleotide that has,

an optimized expression in a recombinant expression host, the method comprising: creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation and iterative manner; whereby these directed evolution methods include the introduction of mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

60. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising: screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host when compared to the expression of a parental polynucleotide from which the library was created.

61. A method for obtaining an immunomodulatory polynucleotide that has an optimized expression in a recombinant expression host, the method comprising: a) creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set; and b) screening a library of non-stochastically generated progeny polynucleotides to identify an optimized non-stochastically generated progeny polynucleotide that has an optimized expression in a recombinant expression host when compared to the expression of a parental polynucleotide from which the library was created; whereby optimization can thus be achieved using one or more of the directed evolution methods as described herein in any combination, permutation, and iterative manner; whereby these directed evolution methods include the introduction of point mutations by non-stochastic methods, including by "gene site saturation mutagenesis" as described herein; and whereby these directed evolution methods also include the introduction mutations by non-stochastic polynucleotide reassembly methods as described herein; including by synthetic ligation polynucleotide reassembly as described herein.

62. The method of any of claims 59-61, wherein the recombinant expression host is a prokaryote.

63. The method of any of claims 59-61, wherein the recombinant expression host is a eukaryote.

64. The method of claim 63, wherein the recombinant expression host is a plant.

65. The method of claim 64, wherein the recombinant expression host is a monocot.

66. The method of claim 64, wherein the recombinant expression host is a dicot.

67. The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "gene site saturation mutagenesis" as described herein.

68. The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to "synthetic ligation polynucleotide reassembly" as described herein.

69. The method of any of claims 1, 3, 5, 53, or 59-61, wherein creating a library of non-stochastically generated progeny polynucleotides from a parental polynucleotide set is comprised of subjecting the parental polynucleotide set to both "gene site saturation mutagenesis" as described herein, and to "synthetic ligation polynucleotide reassembly" as described herein.

70. The method of claim 1, wherein the directed evolution method is synthetic ligation reassembly.

71. The method of claim 1, wherein the directed evolution method is gene site saturated mutagenesis.

72. The method of claim 1, wherein the directed evolution method is non-stochastic ligation reassembly.

73. The method of claim 1, wherein the directed evolution method is exonuclease-mediated reassembly.

74. The method of claim 1, wherein the directed evolution method is end selection.

75. The method of claim 1, wherein the directed evolution method is shuffling.

76. The method of claim 1, wherein the immunomodulatory polynucleofide encodes a cancer antigen.

77. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a bacterial antigen.

78. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a viral antigen.

79. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a parasite antigen.

80. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a self-antigen.

81. The method of claim 1, wherein the immune response is a humoral immune response.

82. The method of claim 1, wherein the immune response is a cellular immune response.

83. The method of claim 1, wherein the immunomodulatory polynucleotide encodes a cytokine.

84. The method of claim 83, wherein the cytokine is IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL14, IL-15, IL-16, IL-17, IL-18, GM-CSF, G-CSF, TNF-α, IFN-α, IFN-γ, or IL-20 (MDA-7).

85. The method as in any of claims 1, 3, or 5, wherein the immune response prior to optimization or following optimization is determined in vitro.

86. The method as in any of claims 1, 3, or 5, wherein the immune response prior to optimization or following optimization is determined in vivo.

L14 ANSWER 16 OF 27 USPATFULL on STN
2002:164401 Human CTLA-4 antibodies and their uses.

Korman, Alan J., Piedmont, CA, UNITED STATES
Halk, Edward L., Sunnyvale, CA, UNITED STATES
Lonberg, Nils, Woodside, CA, UNITED STATES
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APPLICATION: US 2001-948939 A1 20010907 (9)
PRIORITY: US 1999-150452P 19990824 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides novel human sequence antibodies against human CTLA-4 and methods of treating human diseases, infections and other conditions using these antibodies.
CLM What is claimed is:

1. A human sequence antibody that specifically binds to human CTLA-4.

2. A therapeutically-effective human sequence antibody that specifically binds to human CTLA-4.

3. The therapeutically-effective human sequence antibody of claim 2, wherein the antibody binds CTLA-4 on the cell surface of normal human T cells.

4. The therapeutically-effective human sequence antibody of claim 2, wherein the antibody is well-tolerated in a patient.

5. The therapeutically-effective human sequence antibody of claim 2, wherein the T cell subpopulations marked by CD antigens CD4, **CD8**, CD25, and CD69 remain stable during and subsequent to the administration of the antibody.

6. An isolated human sequence antibody that specifically binds to human CTLA-4 which is substantially free of non-immunoglobulin associated human proteins.

7. A composition of polyclonal antibodies comprising a plurality of antibodies according to claim 1.

8. The composition of polyclonal antibodies of claim 7 comprising at least about 10 different antibodies according to claim 1.

9. The composition of polyclonal antibodies of claim 7 comprising at least about 100 different antibodies according to claim 1.

10. The composition of polyclonal antibodies of claim 7 comprising at least about 1000 different antibodies according to claim 1.

11. The human sequence antibody of claim 1, wherein the human antibody blocks binding of human CTLA-4 to B7 ligands.

12. The human sequence antibody of claim 1, wherein the human sequence antibody does not block binding of human CTLA-4 to B7 ligands.

13. The human sequence antibody of claim 1, wherein the antibody binds to human CTLA-4 with an equilibrium association constant (K_a) of at least 10^8 M^{-1}.

14. The human sequence antibody of claim 1, wherein the antibody binds to human CTLA-4 with an equilibrium association constant (K_a) of at least 10^9 M^{-1}.

15. The human sequence antibody of claim 1, wherein the antibody blocks binding of human CTLA-4 to B7 ligands by at least about 20%.

16. The human sequence antibody of claim 1, wherein the antibody blocks binding of human CTLA-4 to B7 ligands by at least about 30%.

17. The human sequence antibody of claim 1, wherein the antibody blocks

binding of human CTLA-4 to B7 ligands by at least about 40%.

18. The human sequence antibody of claim 1, wherein the antibody blocks binding of human CTLA-4 to B7 ligands by at least about 50%.

19. The human sequence antibody of claim 1, wherein the antibody heavy chain is IgG or IgM.

20. The human sequence antibody of claim 19, wherein the IgG antibody heavy chain is IgG_1, IgG_2, IgG_3 or IgG_4.

21. The human sequence antibody of claim 1, wherein the antibody light chain is a kappa light chain.

22. The human sequence antibody of claim 1, wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO: 16 and SEQ ID NO:6, respectively.

23. The human sequence antibody of claim 1, wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO:18 and SEQ ID NO:8, respectively.

24. The human sequence antibody of claim 1, wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids that comprise nucleotide sequences in their variable regions as set forth in SEQ ID NO:22 and SEQ ID NO:12, respectively.

25. A human sequence antibody of claim 1, wherein the human sequence antibody is encoded by heavy chain and light chain variable region amino acid sequences as set for the in SEQ ID NO:17 and SEQ ID NO:7, respectively.

26. A human sequence antibody of claim 1, wherein the human sequence antibody is encoded by heavy chain and light chain variable region amino acid sequences as set for the in SEQ ID NO:19 and SEQ ID NO:9, respectively.

27. A human sequence antibody of claim 1, wherein the human sequence antibody is encoded by heavy chain and light chain variable region amino acid sequences as set for the in SEQ ID NO:23 and SEQ ID NO:13, respectively.

28. The human sequence antibody of claim 1, wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising variable heavy and light chain sequences from V gene segments VH 3-30.3 and VK A-27, respectively.

29. The human sequence antibody of claim 1, wherein the human sequence antibody is encoded by human IgG heavy chain and human kappa light chain nucleic acids comprising variable heavy and light chain sequences from V gene segments VH 3-33 and VK L-15, respectively.

30. The human sequence antibody of claim 1, comprising heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGNNKYYADSVKG (SEQ ID NO:32) and TGWLGPFDY (SEQ ID NO:37), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVGSSYLA (SEQ ID NO:24), GAFSRAT (SEQ ID NO:29), and QQYGSSPWT (SEQ ID NO:35), respectively.

31. The human sequence antibody of claim 1, comprising heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGSNKHYADSVKG (SEQ ID NO:33) and TGWLGPFDY (SEQ ID NO:38), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVSSSFLA (SEQ ID NO:25), GASSRAT (SEQ ID NO:30), and QQYGSSPWT (SEQ ID NO:35), respectively.

32. The human sequence antibody of claim 1, comprising heavy chain CDR1, CDR2, and CDR3 sequences, SYGMH (SEQ ID NO:28), VIWYDGSNKYYADSVKG (SEQ ID NO:34) and APNYIGAFDV (SEQ ID NO:39), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQGISSWLA (SEQ ID NO:26), AASSLQS (SEQ ID NO:31), and QQYNSYPPT (SEQ ID NO:36), respectively.

33. The human sequence antibody of claim 1, wherein said human sequence antibody is produced by a transgenic non-human animal.

34. The human sequence antibody of claim 33, wherein said transgenic non-human animal is a mouse.

35. The human sequence antibody of claim 1, wherein the human sequence antibody is a Fab fragment.

36. A polyvalent complex comprising at least two human sequence antibodies each of which specifically binds to human CTLA-4.

37. The polyvalent complex of claim 36, wherein the two different antibodies are linked to each other covalently.

38. The polyvalent complex of claim 36, wherein the two different antibodies are linked to each other non-covalently.

39. A nucleic acid encoding a heavy chain of a human sequence antibody.

40. The nucleic acid of claim comprising the nucleotide sequence as set forth in SEQ ID NO:1.

41. A transgenic non-human animal having a genome comprising a human sequence heavy chain transgene and a human sequence light chain transgene, which animal has been immunized with a human CTLA-4, or a fragment or an analog thereof, whereby the animal expresses human sequence antibodies to the human CTLA-4.

42. The transgenic non-human animal of claim 41, wherein the transgenic non-human animal is a transgenic mouse.

43. The transgenic mouse of claim 42 comprising HCo7 or HCo 12.

44. A cell line comprising a B cell obtained from a transgenic non-human animal having a genome comprising a human sequence heavy chain transgene and a human sequence light chain transgene, wherein the hybridoma produces a human sequence antibody that specifically binds to human CTLA-4.

45. The cell line of claim 44, wherein the cell line is a hybridoma.

46. A hybridoma secreting a human sequence antibody that specifically binds human CTLA-4 or binding fragment thereof, wherein the antibody is selected from the group consisting of: a human sequence antibody comprising heavy chain heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGNNKYYADSVKG (SEQ ID NO:32) and TGWLGPFDY (SEQ ID NO:37), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVGSSYLA (SEQ ID NO:24), GAFSRAT (SEQ ID NO:29), and QQYGSSPWT (SEQ ID NO:35), respectively, and heavy chain and light chain variable region amino acid sequences as set forth in SEQ ID NO:17 and SEQ ID NO:7, respectively, a human sequence antibody comprising heavy chain CDR1, CDR2, and CDR3 sequences, SYTMH (SEQ ID NO:27), FISYDGSNKHYADSVKG (SEQ ID NO:33) and TGWLGPFDY (SEQ ID NO:38), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQSVSSSFLA (SEQ ID NO:25), GASSRAT (SEQ ID NO:30), and QQYGSSPWT (SEQ ID NO:35), respectively, and heavy chain and light chain variable region amino acid sequences as set forth in SEQ ID NO:19 and SEQ ID NO:9, respectively, and a human sequence antibody of claim 1, comprising heavy chain CDR1, CDR2, and CDR3 sequences, SYGMH (SEQ ID NO:28), VIWYDGSNKYYADSVKG (SEQ ID NO:34) and

APNYIGAFDV (SEQ ID NO:39), respectively, and light chain CDR1, CDR2, and CDR3 sequences, RASQGISSWLA (SEQ ID NO:26), AASSLQS (SEQ ID NO:31), and QQYNSYPPT (SEQ ID NO:36), respectively, and heavy chain and light chain variable region amino acid sequences as set forth in SEQ ID NO:23 and SEQ ID NO:13, respectively.

47. A pharmaceutical composition comprising a human sequence antibody of claim and a pharmaceutically acceptable carrier.

48. The pharmaceutical composition of claim 47, further comprising an agent effective to induce an immune response against a target antigen.

49. The pharmaceutical composition of claim 47, further comprising a chemotherapeutic agent.

50. The pharmaceutical composition of claim 47, further comprising an antibody to an immunosuppressive molecule.

51. A method for inducing, augmenting or prolonging an immune response to an antigen in a patient, comprising administering a therapeutically effective amount of a pharmaceutical composition of claim 47, wherein the pharmaceutical composition blocks binding of human CTLA-4 to human B7 ligands.

52. The method of claim 51, wherein the antigen is a tumor antigen or an antigen from a pathogen.

53. The method of claim 52, wherein the patient is also treated with a bispecific antibody, said bispecific antibody comprising an antibody sequence having an affinity for an antigen from a tumor or a pathogen.

54. The method of claim 52, wherein the pathogen is a virus, a bacterium, a fungus or a parasite.

55. The method of claim 54, wherein the pathogen is **HIV**.

56. The method of claim 51, further comprising administering the antigen, or a fragment or an analog thereof, to the patient, whereby the antigen in combination with the human sequence antibody induces, augments or prolongs the immune response.

57. The method of claim 56, wherein the antigen is a tumor antigen or an antigen from a pathogen.

58. The method of claim 57, wherein the tumor antigen is telomerase.

59. The method of claim 56, wherein the antigen is a component of an amyloid formation in the patient.

60. The method of claim 56, wherein the patient is suffering from Alzheimer's disease and the antigen is AB peptide.

61. The method of claim 51, further comprising administering a cytokine to the patient.

62. A method of **suppressing** an immune response in a patient, comprising administering to the patient a therapeutically effective dosage of a polyvalent preparation comprising at least two human sequence antibodies to human CTLA-4 linked to each other.

63. A method of **suppressing** an immune response in a patient, comprising administering to the patient a therapeutically effective dosage of a polyclonal preparation comprising at least two human sequence antibodies to human CTLA-4.

64. A method of treating an autoimmune disease in a subject caused or

exacerbated by increased activity of T cells consisting of administering a theraputically effective amount of a pharmaceutical composition of claim 47 to the subject.

65. A method of treating cancer in a subject consisting of administering a therapeutically effective amount of a pharmaceutical composition of claim 47 to the subject.

66. The method of claim 65, wherein the cancer is prostate cancer, melanoma, or epithelial cancer.

67. The method of claim 65, further comprising a vaccine.

68. The method of claim 67, wherein the vaccine is a tumor cell vaccine, a GM-CSF-modified tumor cell vaccine, or an antigen-loaded dendritic cell vaccine.

L14 ANSWER 17 OF 27 USPATFULL on STN
2002:67199 BAFF, **inhibitors** thereof and their use in the modulation of B-cell response.
Browning, Jeffrey, Brookline, MA, UNITED STATES
Ambrose, Christine, Reading, MA, UNITED STATES
MacKay, Fabienne, Vaucluse, AUSTRALIA
Tschopp, Jurg, Epalinges, SWITZERLAND
Schneider, Pascal, Epalinges, SWITZERLAND
US 2002037852 A1 20020328
APPLICATION: US 2001-911777 A1 20010724 (9)
PRIORITY: WO 2000-US1788 20000125
US 1999-117169P 19990125 (60)
US 1999-143228P 19990709 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides methods for treating or preventing disorders associated with expression of BAFF comprising BAFF and fragments thereof, antibodies, agonists and antagonists.
CLM What is claimed is:
1. A method of stimulating B-cell growth in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; and (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule.

2. A method of stimulating immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule.

3. A method of co-stimulating B-cell growth and immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; and (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule.

4. A method of stimulating dendritic cell-induced B-cell growth and maturation comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or

an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; and (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule.

5. The method according to claims 1-4 wherein the BAFF ligand is a soluble BAFF ligand.

6. The method according to claim 5 wherein the soluble BAFF ligand is a recombinant BAFF ligand.

7. The method according to claims 1-4 wherein the anti-CD40 molecule is a monoclonal antibody.

8. The method according to claims 1-4 wherein the animal is of mammalian origin.

9. The method according to claim 8 wherein the mammal is human.

10. A method of **inhibiting** B-cell growth in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a anti-BAFF ligand molecule or an active fragment thereof; (b) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (c) an antibody specific for BAFF ligand or an active fragment thereof; and (d) an antibody specific for BAFF ligand receptor or an epitope thereof.

11. A method of **inhibiting** immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a anti-BAFF ligand molecule or an active fragment thereof; (b) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (c) an antibody specific for BAFF ligand or an active fragment thereof; and (d) an antibody specific for BAFF ligand receptor or an epitope thereof.

12. A method of co-**inhibiting** B-cell growth and immunoglobulin production in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a anti-BAFF ligand molecule or an active fragment thereof; (b) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (c) an antibody specific for BAFF ligand or an active fragment thereof; and (d) an antibody specific for BAFF ligand receptor or an epitope thereof.

13. A method of **inhibiting** dendritic cell-induced B-cell growth and maturation in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a anti-BAFF ligand molecule or an active fragment thereof; (b) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (c) an antibody specific for BAFF ligand or an active fragment thereof; and (d) an antibody specific for BAFF ligand receptor or an epitope thereof.

14. The method according to claims 10-13, wherein the anti-BAFF ligand is soluble.

15. The method according to claim 14, wherein the soluble anti-BAFF ligand is a recombinant anti-BAFF ligand.

16. The method according to claims 10-13, wherein the anti-BAFF antibody is a monoclonal antibody. The method according to claims 10-13, wherein the anti-BAFF receptor antibody is a monoclonal antibody.

17. A method of treatment of an autoimmune disease comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and

an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule; (e) a anti-BAFF ligand molecule or an active fragment thereof; (f) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (g) an antibody specific for BAFF ligand or an active fragment thereof; and (h) an antibody specific for BAFF ligand receptor or an epitope thereof.

18. A method of treating a disorder related to BAFF-ligand comprising the steps of: (a) introducing into a desired cell a therapeutically effective amount of a vector containing a gene encoding for a BAFF-related molecule; and (b) expressing said gene in said cell.

19. The method according to claim 18, wherein the BAFF-related molecule is selected from the group consisting of: (a) a BAFF ligand or an active fragment thereof; (b) a BAFF ligand or an active fragment thereof and an anti-T antibody; (c) a BAFF ligand or an active fragment thereof and a CD40 ligand; (d) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule; (e) a anti-BAFF ligand molecule or an active fragment thereof; (f) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (g) an antibody specific for BAFF ligand or an active fragment thereof; and (h) an antibody specific for BAFF ligand receptor or an epitope thereof.

20. The method according to claims 17-19 , wherein the BAFF ligand is a soluble BAFF ligand.

21. The method according to claim 20, wherein the soluble BAFF ligand is a recombinant BAFF ligand.

22. The method according to claims 17-19, wherein the anti-CD40 molecule is a monoclonal antibody.

23. The method according to claims 17-19, wherein the anti-BAFF ligand is soluble.

24. The method according to claim 23, wherein the soluble anti-BAFF ligand is a recombinant anti-BAFF ligand.

25. The method according to claims 17-19, wherein the anti-BAFF antibody is a monoclonal antibody.

26. The method according to claims 17-19, wherein the anti-BAFF receptor antibody is a monoclonal antibody.

27. A method of inducing cell death comprising the administration of an agent capable of interfering with the binding of a BAFF-ligand to a receptor.

28. A method of treating, **suppressing** or altering an immune response involving a signaling pathway between a BAFF-ligand and its receptor comprising the step of administering an effective amount of an agent capable of interfering with the association between the BAFF-ligand and its receptor.

29. A method of **inhibiting** inflammation comprising the step of administering a therapeutically effective amount of an antibody specific for a BAFF-ligand or an active fragment thereof.

30. A method of **inhibiting** inflammation comprising the step of administering a therapeutically effective amount of an antibody specific for a BAFF-ligand receptor or an epitope thereof.

31. A method of regulating hematopoietic cell development comprising the step of administering a therapeutically effective amount of a BAFF-ligand or an active fragment thereof.

32. A method of treating, **suppressing** or altering an immune response involving a signaling pathway between a BAFF-ligand and its receptor comprising the step of administering an effective amount of an agent capable of interfering with the association between the BAFF-ligand and its receptor.

33. A method of treating hypertension in an animal comprising the step of administering a therapeutically effective amount of a B-cell growth **inhibitor**.

34. The method according to claim 33, wherein the B-cell growth **inhibitor** is selected from the group consisting of: (e) (a) a anti-BAFF ligand molecule or an active fragment thereof; (f) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (g) an antibody specific for BAFF ligand or an active fragment thereof; and (h) an antibody specific for BAFF ligand receptor or an epitope thereof.

35. The method according to claim 34, wherein the anti-BAFF ligand is soluble.

36. The method according to claim 35, wherein the soluble anti-BAFF ligand is a recombinant anti-BAFF ligand.

37. The method according to claim 34, wherein the anti-BAFF antibody is a monoclonal antibody.

38. The method according to claim 34, wherein the anti-BAFF receptor antibody is a monoclonal antibody.

39. The method according to claim 34, wherein the animal is of mammalian origin.

40. The method according to claim 39, wherein the mammal is human.

41. A method of treating hypertension in an animal comprising the step of administering a therapeutically effective amount of a co-**inhibitor** of B-cell growth and immunoglobulin secretion.

42. A method of treating cardiovascular disorders in an animal comprising the step of administering a therapeutically effective amount of a B-cell growth **inhibitor**.

43. A method of treating cardiovascular disorders in an animal comprising the step of administering a therapeutically effective amount of a co-**inhibitor** of B-cell growth and immunoglobulin production.

44. A method of treating renal disorders in an animal comprising the step of administering a therapeutically effective amount of a B -cell growth **inhibitor**.

45. A method of treating renal disorders in an animal comprising the step of administering a therapeutically effective amount of a co-**inhibitor** of B-cell growth and immunoglobulin production

46. A method of treating B-cell lympho-proliferate disorders comprising the step of administering a therapeutically effective amount of a B-cell growth **inhibitor**.

47. A method of stimulating B-cell production in the treatment of immunosuppressive diseases comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (e) a BAFF ligand or an active fragment thereof; (f) a BAFF ligand or an active fragment thereof and an anti-T antibody; (g) a BAFF ligand or an active fragment thereof and a CD40 ligand; (h)

a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule; (i) a anti-BAFF ligand molecule or an active fragment thereof; (j) a recombinant, inoperative BAFF ligand molecule or an active fragment thereof; (k) an antibody specific for BAFF ligand or an active fragment thereof; and (l) an antibody specific for BAFF ligand receptor or an epitope thereof.

48. A method of stimulating B-cell production in the treatment of an immunosuppressive disease comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of: (i) a BAFF ligand or an active fragment thereof; (j) a BAFF ligand or an active fragment thereof and an anti-T antibody; (k) a BAFF ligand or an active fragment thereof and a CD40 ligand; (l) a BAFF ligand or an active fragment thereof and an anti-CD40 ligand molecule; (m) a anti-BAFF ligand molecule or an active fragment thereof; (n) a recombinant, inoperative BAFF ligand molecule or an active fragment (o) an antibody specific for BAFF ligand or an active fragment thereof; and

49. A method according to claim 48 wherein the immunosuppressive disease is **HIV**.

50. A method according to claim 49 wherein the immunosuppressive disease is associated with an organ transplantation.

L14 ANSWER 18 OF 27 USPATFULL on STN
2002:48017 NUCLEIC ACID ENCODING FELINE **CD86**.
COLLISSON, ELLEN W., COLLEGE STATION, TX, UNITED STATES
HASH, STEPHEN M., AUSTIN, TX, UNITED STATES
CHOI, INSOO, COLLEGE STATION, TX, UNITED STATES
US 2002028208 A1 20020307
APPLICATION: US 1999-303510 A1 19990430 (9)
PRIORITY: US 1998-83869P 19980501 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides isolated and purified DNA encoding feline **CD80** (B7-1) ligand, feline **CD86** (B7-2) ligand, feline CD28 receptor, or feline CTLA-4 (CD152) receptor, as well as vectors comprising nucleic acid encoding feline **CD80**, feline **CD86**, feline CD28, or feline CTLA-4. The present invention provides a host cells transformed with **CD80**-encoding vectors, **CD86**-encoding vectors, CD28-encoding vectors, or CTLA-4-encoding vectors. The invention provides polypeptides encoded by the nucleic acid of feline **CD80**, feline **CD86**, feline CD28, or feline CTLA-4.

The present invention provides a vaccine comprising an effective amount of polypeptides encoded by the nucleic acid of feline **CD80**, feline **CD86**, feline CD28, or feline CTLA-4. The present invention also provides vaccines which further comprise immunogens derived from pathogens. The invention provides for vaccines capable of enhancing an immune response. The invention also provides for vaccines capable of **suppressing** and immune response.
CLM What is claimed is:
1. An isolated nucleic acid encoding a feline **CD80** ligand or a feline soluble **CD80** ligand.

2. An isolated nucleic acid encoding a feline **CD86** ligand or a feline soluble **CD86** ligand.

3. An isolated nucleic acid encoding a feline CD28 receptor or a feline soluble CD28 receptor.

4. An isolated nucleic acid encoding a feline CTLA-4 receptor or a feline soluble CTLA-4 receptor.

5. The nucleic acid of claim 1, wherein the feline **CD80** ligand has the sequence shown in FIG. 1A beginning with methionine and ending with threonine(Sequence ID NO: 1).

6. The nucleic acid of claim 2, wherein the feline **CD86** ligand has the sequence shown in FIG. 3A beginning with methionine and ending with isoleucine(Sequence ID NO: 5).

7. The nucleic acid of claim 3, wherein the feline **CD86** receptor shown in FIG. 4A has the sequence beginning with methionine and ending with serine (Sequence ID NO: 7).

8. The nucleic acid of claim 4, wherein the feline CTLA-4 receptor has the sequence shown in FIG. 5A beginning with methionine and ending with asparagine(Sequence I.D. NO: 9).

9. The nucleic acid of any of claim 1-4, wherein the nucleic acid is DNA or RNA.

10. The nucleic acid of claim 9, wherein the DNA is cDNA or genomic DNA.

11. An oligonucleotide of at least 12 nucleotides which has a sequence complementary to a sequence uniquely present in the nucleic acid of any of claim 1-4.

12. The oligonucleotide of claim 11 which is at least 15 or 16 nucleotides in length.

13. The oligonucleotide of claim 11 or 12, wherein the oligonucleotide is detectably labeled.

14. The oligonucleotide of claim 13, wherein the detectable label comprises a radioisotope, a fluorophor, or biotin.

15. The oligonucleotide of claim 11 or 12, wherein the oligonucleotide is selectively methylated.

16. A vector comprising the nucleic acid of claim 1.

17. The plasmid vector of claim 16 designated PSI-B7-1/871-35 (ATCC Accession No. 209817).

18. A vector comprising the nucleic acid of claim 2.

19. The plasmid vector of claim 18 designated B7-2#19-2/011298 (ATCC Accession No. 209821).

20. A vector comprising the nucleic acid of claim 3.

21. The plasmid vector of claim 20 designated PSI-CD28 #7/100296 (ATCC Accession No. 209819).

22. A vector comprising the nucleic acid of claim 4.

23. The plasmid vector of claim 22 designated CTLA-4#1/091997 (ATCC Accession No. 209820).

24. The vector of any of claim 16-23, comprising a promoter operably linked to the nucleic acid.

25. A host cell which comprises a vector of any of claim 16-24.

26. The host cell of claim 25, wherein the host cell is a eukaryotic or a prokaryotic cell.

27. The host cell of claim 26, wherein the host cell is selected from

the group consisting of: E. coli, yeast, COS cells, PC12 cells, CHO cells, and GH4C1 cells.

28. A polypeptide encoded by the nucleic acid of claim 1.

29. A polypeptide encoded by the nucleic acid of claim 2.

30. A polypeptide encoded by the nucleic acid of claim 3.

31. A polypeptide encoded by the nucleic acid of claim 4.

32. A method of producing the polypeptide of any of claim 28-31 which comprising culturing a host cell which expresses the polypeptide and recovering the polypeptide so produced.

33. A vaccine comprising an effective amount of a polypeptide of any of claim 28-30 and a suitable carrier.

34. A vaccine of claim 33, wherein the effective amount is an amount from about 0.01 mg to about 100 mg per dose.

35. A vaccine of claim 33, wherein the effective amount is an amount from about 0.25 mg/kg weight body of a feline/day to about 25 mg/kb weight of a feline/day.

36. A vaccine of claim 33-35 which further comprises an immunogen derived from a pathogen.

37. A vaccine of claims 36, wherein the pathogen in a feline pathogen a rabies virus, chlamydia, Toxoplasmosis gondii, Dirofilaria immitis, a flea, or a bacterial pathogen.

38. A vaccine of claim 37 wherein the feline pathogen is feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), feline infectious peritonitis virus (FIP), feline panleukopenia virus, feline calicivirus, feline reovirus type 3, feline rotavirus, feline coronavirus, feline syncytial virus, feline sarcoma virus, feline herpesvirus, feline Borna disease virus, or a feline parasite.

39. A method of inducing immunity in a feline which comprises administering to the feline a dose of a vaccine of any of claim 36-38.

40. A method of enhancing an immune response in a feline which comprises administering to the feline a dose of a vaccine of any of claim 33-38.

41. The method of claim 39 or 40 wherein the vaccine is administered subcutaneously, intramuscularly, systemically, topically, or orally.

42. A method for **suppressing** an immune response in a feline which comprises administering to the feline an effective immune response **suppressing** amount of a polypeptide of claim 31.

43. A method for **suppressing** an immune response in a feline which comprises administering to the feline an effective immune response **suppressing** amount of a soluble polypeptide of claim 28-30.

44. A method of claim 42 or 43 wherein the amount is from about 0.25 mg/kg body weight/day to about 25 mg/kb body weight/day.

45. A method of claim 42 or 43 wherein the feline is suffering from an autoimmune disease or is the recipient of a tissue or organ transplant.

L14 ANSWER 19 OF 27 USPATFULL on STN
2001:229421 T-CELL CLONES EXPRESSING ANTI-**HIV**-1 FACTORS.
SAHA, KUNAL, HILLIARD, OH, United States

US 2001051373 A1 20011213
APPLICATION: US 1999-369333 A1 19990806 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Certain clones of CD4+ cells from an **HIV**-infected patient who has not progressed to AIDS exhibit the ability to **inhibit HIV** replication at a stage subsequent to entry of the **HIV**-1 virus into the cell and integration of viral cDNA into the cellular genome. These cells secrete a **soluble factor** different from known cytokines and chemokines that can, **inhibit** infection of lymphocytes by SI **HIV**-1.
CLM What is claimed is:
1. A CD4+ T-cell clone capable of producing **soluble factors** other than IFN-α that can **inhibit** replication of SI **HIV**-1 in infected T cells.

2. The CD4+ T-cell clone of claim 1 wherein said T-cell clone is derived from an **HIV**-1 infected nonprogressor.

3. The T-cell clone of claim 1 wherein said T-cell clone is NP1-2 (ATCC . . .).

4. The T-cell clone of claim 1 wherein said T-cell clone is NP1-3 (ATCC . . .).

5. The T-cell clone of claim 1 wherein said T-cell clone is NP1-5 (ATCC . . .).

L14 ANSWER 20 OF 27 USPATFULL on STN
2001:188209 Vaccine and immunotherapy for solid nonlymphoid tumor and related immune dysregulation.
Barbera-Guillem, Emilio, Powell, OH, United States
US 2001033839 A1 20011025
APPLICATION: US 2001-835759 A1 20010416 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Provided are vaccines, methods of making the vaccines and methods for administering the vaccines for immunotherapy of an individual bearing, or at risk for developing, solid nonlymphoid tumor. The vaccine comprises an immunotherapeutic composition and tumor-associated antigen, and may further comprise one or more of an immunomodulator or a pharmaceutically acceptable carrier. A method of immunotherapy of an individual comprises administering to the individual an amount of the vaccine effective to **suppress** a TH2 response, and to induce a TH1 response against solid nonlymphoid tumor, in an individual having a TH2/TH1 imbalance.
CLM What is claimed is:
1. A vaccine for **suppressing** a TH2 response and for inducing a cell mediated immune response comprising a TH1 response in an individual having a TH2/TH1 imbalance, the vaccine comprising: an immunotherapeutic composition for effecting B cell depletion; and tumor-associated antigen capable of inducing a cell mediated immune response comprising a TH1 response.

2. The vaccine according to claim 1, further comprising a component selected from the group consisting of an immunomodulator for inducing a cell mediated immune response comprising a TH1 response, a pharmaceutically acceptable carrier, and a combination thereof.

3. The vaccine according to claim 1, wherein the immunotherapeutic composition is contained in a solid phase implant for delivery of the immunotherapeutic composition.

4. The vaccine according to claim 1, wherein the immunotherapeutic composition further comprises an anti-B cell agent.

5. The vaccine according to claim 1, wherein the immunotherapeutic composition comprises an affinity ligand having binding specificity for a determinant selected from the group consisting of CD19, CD20, CD21, CD22 (also known as LL2), CDIM, and Lym-1.

6. The vaccine according to claim 1, wherein the immunotherapeutic composition comprises cobra venom factor.

7. The vaccine according to claim 1, wherein the TH2/TH1 imbalance is mediated by a disease process comprising a pro-tumor immune response.

8. The vaccine according to claim 1, wherein the TH2/TH1 imbalance is mediated by a disease process comprising a pro-tumor immune response and solid nonlymphoid tumor.

9. A vaccine useful for the treatment or prevention of solid nonlymphoid tumor in an individual, the vaccine comprising: an immunotherapeutic composition for effecting B cell depletion; and tumor-associated antigen capable of inducing a cell mediated immune response comprising a TH1 response.

10. The vaccine according to claim 9, further comprising a component selected from the group consisting of an immunomodulator for inducing a cell mediated immune response comprising a TH1 response, a pharmaceutically acceptable carrier, and a combination thereof.

11. The vaccine according to claim 9, wherein the immunotherapeutic composition further comprises an anti-B cell agent.

12. The vaccine according to claim 9, wherein the immunotherapeutic composition comprises an affinity ligand having binding specificity for a determinant selected from the group consisting of CD19, CD20, CD21, CD22 (also known as LL2), CDIM, and Lym-1.

13. The vaccine according to claim 9, wherein the immunotherapeutic composition comprises cobra venom factor.

14. A composition comprising micelles comprised of tumor-associated antigen, wherein the composition is substantially free of solubilizing agents, wherein the composition is substantially free of oil, wherein the tumor-associated antigen comprises tumor cells lysed by a freeze-thaw process, and wherein the composition further comprises a pharmaceutically acceptable carrier.

15. The composition according to claim 14, wherein the composition comprises micelles of tumor-associated antigen, wherein the micelles comprise diameters that range from about 0.5 microns in diameter to diameters smaller than 0.5 microns.

16. The composition according to claim 14, wherein the composition is capable of, inducing an immunologic cross-protection against solid nonlymphoid tumors selected from the group consisting of solid nonlymphoid tumors of the same tissue but different origin than the solid nonlymphoid tumor from which the composition is produced, solid nonlymphoid tumors of different tissues than the solid nonlymphoid tumor from which the composition is produced, and a combination thereof.

17. A method for immunotherapy of a TH2/TH1 imbalance in an individual comprising administering to the individual a vaccine in an amount effective to reduce a TH2 response, and in an amount effective to induce a cell mediated immune response comprising a TH1 response against solid nonlymphoid tumor, wherein the vaccine comprises: an immunotherapeutic composition for effecting B cell depletion; and tumor-associated antigen capable of inducing a cell mediated immune response comprising a TH1 response; wherein the TH2/TH1 imbalance is mediated by a disease process selected from the group consisting of a pro-tumor immune

response, solid nonlymphoid tumor, and a combination thereof.

18. The method according to claim 17, wherein the vaccine is administered to the individual by administering a priming dose comprising the immunotherapeutic composition, and administering an immunizing dose comprising tumor-associated antigen.

19. The method according to claim 17, wherein the vaccine further comprises a component selected from the group consisting of an immunomodulator for inducing a TH1 response, a pharmaceutically acceptable carrier, and a combination thereof.

20. The method according to claim 17, wherein the immunotherapeutic composition further comprises an anti-B cell agent.

21. The method according to claim 17, wherein the immunotherapeutic composition comprises an affinity ligand having binding specificity for a determinant selected from the group consisting of CD19, CD20, CD21, CD22 (also known as LL2), CDIM, and Lym-1.

22. The method according to claim 17, wherein the TH2 response reduced comprises a humoral immune response against shed tumor antigen.

23. The method according to claim 17, wherein the cell mediated immune response induced comprises a TH1 response against tumor-associated antigen.

24. The method according to 17, wherein the immunotherapeutic composition of the vaccine is administered to the individual at a time selected from the group consisting of before tumor-associated antigen of the vaccine is administered to the individual, simultaneous with the administration of tumor-associated antigen of the vaccine to the individual, subsequent to administration of tumor-associated antigen of the vaccine to the individual, and a combination thereof.

25. The method according to 19, wherein the vaccine further comprises an immunomodulator, and the immunomodulator is administered to the individual at a time selected from the group consisting of before tumor-associated antigen of the vaccine is administered to the individual, simultaneous with the administration of tumor-associated antigen of the vaccine to the individual, subsequent to administration of tumor-associated antigen of the vaccine to the individual, and a combination thereof.

26. The method according to 17, wherein the vaccine is administered parenterally.

27. A method for immunotherapy of an individual for treatment or prevention of solid nonlymphoid tumor, the method comprising administering to the individual a vaccine in an amount effective to reduce a TH2 response, and in an amount effective to induce a cell mediated immune response against solid nonlymphoid tumor, wherein the vaccine comprises: an immunotherapeutic composition for effecting B cell depletion; and tumor-associated antigen capable of inducing a cell mediated immune response comprising an immune response selected from the group consisting of a TH1 response, a cytotoxic **CD8+ T cell** response, and a combination thereof.

28. The method according to claim 27, wherein the vaccine is administered to the individual by administering a priming dose comprising the immunotherapeutic composition, and administering an immunizing dose comprising tumor-associated antigen.

29. The method according to claim 27, wherein the vaccine further comprises a component selected from the group consisting of an immunomodulator, a pharmaceutically acceptable carrier, and a

combination thereof.

30. The method according to claim 27, wherein the immunotherapeutic composition further comprises an anti-B cell agent.

31. The method according to claim 27, wherein the immunotherapeutic composition comprises an affinity ligand having binding specificity for a determinant selected from the group consisting of CD19, CD20, CD21, CD22 (also known as LL2), CDIM, and Lym-1.

32. The method according to claim 27, wherein the TH2 response reduced comprises a humoral immune response against shed tumor antigen.

33. The method according to claim 27, wherein the cell mediated immune response induced comprises a cell mediated immune response against tumor-associated antigen.

34. The method according to 27, wherein the immunotherapeutic composition of the vaccine is administered to the individual at a time selected from the group consisting of before tumor-associated antigen of the vaccine is administered to the individual, simultaneous with the administration of tumor-associated antigen of the vaccine to the individual, subsequent to administration of tumor-associated antigen of the vaccine to the individual, and a combination thereof.

35. The method according to 28, wherein the vaccine further comprises an immunomodulator, and the immunomodulator is administered to the individual at a time selected from the group consisting of before tumor-associated antigen of the vaccine is administered to the individual, simultaneous with the administration of tumor-associated antigen of the vaccine to the individual, subsequent to administration of tumor-associated antigen of the vaccine to the individual, and a combination thereof.

36. The method according to 27, wherein the vaccine is administered parenterally.

37. A method for immunotherapy of an individual for treatment or prevention of solid nonlymphoid tumor, the method comprising administering to the individual a vaccine comprising: a priming dose comprised of a composition selected from the group consisting of an immunotherapeutic composition, anti-CD4 monoclonal antibody, and a combination thereof; and an immunizing dose comprised of tumor-associated antigen capable of inducing a cell mediated immune response comprising an immune response selected from the group consisting of a TH1 response, a cytotoxic **CD8+ T cell** response, and a combination thereof.

38. The method according to claim 37, wherein the vaccine further comprises a component selected from the group consisting of an immunomodulator, a pharmaceutically acceptable carrier, and a combination thereof.

39. The method according to claim 37, wherein the priming dose is administered as a solid phase implant containing the composition comprising the priming dose for delivery to the individual.

40. The method according to claim 37, wherein the priming dose comprises a composition comprising an immunotherapeutic composition, and the immunotherapeutic composition comprises an affinity ligand having binding specificity for a determinant selected from the group consisting of CD19, CD20, CD21, CD22 (also known as LL2), CDIM, and Lym-1.

41. The method according to claim 37, wherein the immunizing dose is administered at a time following administration of the primary dose to the individual.

42. The method according to claim 37, wherein the priming dose comprises a composition comprising anti-CD4 monoclonal antibody, and wherein the immunizing dose induces a cell mediated immune response comprising a cytotoxic **CD8+ T cell** response.

43. A method of making the vaccine according to claim 1, the method comprising combining an immunotherapeutic composition, in an amount effective to deplete B cells, with tumor-associated antigen, in an amount effective for inducing a cell mediated immune response comprising a TH1 response, in making the vaccine.

44. A method of making the vaccine according to claim 2, the method comprising combining an immunotherapeutic composition, in an amount effective to deplete B cells, with tumor-associated antigen, in an amount effective for inducing a cell mediated immune response comprising a TH1 response, with an immunomodulator in an amount effective for inducing a cell mediated immune response comprising a TH1 response, in making the vaccine.

45. A method of making the vaccine according to claim 2, the method comprising combining an immunotherapeutic composition, in an amount effective to deplete B cells, with tumor-associated antigen, in an amount effective for inducing a cell mediated immune response comprising a TH1 response, with a pharmaceutically acceptable carrier, in making the vaccine.

46. A method of making the vaccine according to claim 2, the method comprising combining an immunotherapeutic composition, in an amount effective to deplete B cells, with tumor-associated antigen, in an amount effective for inducing a cell mediated immune response comprising a TH1 response, with an immunomodulator in an amount effective for inducing a cell mediated immune response comprising a TH1 response, with a pharmaceutically acceptable carrier, in making the vaccine.

47. A method of making the vaccine according to claim 9, the method comprising combining an immunotherapeutic composition, in an amount effective to deplete B cells, with tumor-associated antigen, in an amount effective for inducing a cell mediated immune response comprising a TH1 response, in making the vaccine.

48. A method of making the vaccine according to claim 10, the method comprising combining an immunotherapeutic composition, in an amount effective to deplete B cells, with tumor-associated antigen, in an amount effective for inducing a cell mediated immune response comprising a TH1 response, with an immunomodulator in an amount effective for inducing a cell mediated immune response comprising a TH1 response, in making the vaccine.

49. A method of making the vaccine according to claim 10, the method comprising combining an immunotherapeutic composition, in an amount effective to deplete B cells, with tumor-associated antigen, in an amount effective for inducing a cell mediated immune response comprising a TH1 response, with a pharmaceutically acceptable carrier, in making the vaccine.

50. A method of making the vaccine according to claim 10, the method comprising combining an immunotherapeutic composition, in an amount effective to deplete B cells, with tumor-associated antigen, in an amount effective for inducing a cell mediated immune response comprising a TH1 response, with an immunomodulator in an amount effective for inducing a cell mediated immune response comprising a TH1 response, with a pharmaceutically acceptable carrier, in making the vaccine.

51. A method of making the composition according to 14, the method comprising: (a) forming a pellet of tumor cells; (b) exposing the

pelleted tumor cells to a plurality of freeze/thaw cycles to disrupt the cells; (c) resuspending the disrupted cells, and any whole cells that may still be present, in a pharmaceutically acceptable carrier in forming a suspension; (d) filtering the suspension through a filter to remove any components greater than or equal to about 1 micron that may be present in forming a filtered tumor cell lysate; and (e) extruding the filtered tumor cell lysate through a filter comprising pores of a size sufficient to induce formation of micelles in forming a composition comprising micelles comprised of tumor-associated antigen.

52. The method according to claim 51, wherein in forming a filtered tumor cell lysate, the suspension is passed through a first filter comprising pores of a size of greater than 1 micron but less than about 150 microns, and resultant filtrate is then flowed through a second filter comprising pores of a size of about 1 micron in forming a filtered tumor cell lysate.

53. The method according to claim 52, wherein the first filter comprises pores of a size of about 100 microns.

54. The method according to claim 51, wherein the filtered tumor lysate is extruded through a filter comprising pores of a size in the range of from about 0.2 microns to about 0.7 microns.

55. The method according to claim 51, wherein the filtered tumor lysate is extruded through a filter comprising pores of a size of about 0.5 microns.

56. The method according to claim 51, wherein the plurality of freeze/thaw cycles comprises a number of cycles in the range of from about 2 to about 10.

57. A method for priming the immune system of an individual, the method comprises: administering to the individual a priming dose, wherein the priming dose comprises a composition selected from the group consisting of an immunotherapeutic composition, anti-CD4 monoclonal antibody, and a combination thereof; wherein the priming dose is administered in an amount effective to modulate the individual's immune system to respond with induction of a cell mediated immune response upon administration of an immunizing dose of tumor-associated antigen to the individual.

58. The method according to claim 57, wherein the priming dose further comprises a component selected from the group consisting of an immunomodulator, a pharmaceutically acceptable carrier, and a combination thereof.

59. The method according to claim 57, wherein the priming dose is administered as a solid phase implant containing the composition comprising the priming dose for delivery to the individual.

60. The method according to claim 58, wherein the priming dose is administered as a solid phase implant.

61. The method according to claim 57, wherein the priming dose comprises a composition comprising an immunotherapeutic composition, and the immunotherapeutic composition comprises an affinity ligand having binding specificity for a determinant selected from the group consisting of CD19, CD20, CD21, CD22 (also known as LL2), CDIM, and Lym-1.

62. The method according to claim 57, wherein the priming dose comprises a composition comprising anti-CD4 monoclonal antibody, and wherein the priming dose modulates the individual's immune system to respond with induction of a cell mediated immune response comprising a cytotoxic **CD8+ T cell** response.

63. The method according to claim 58, wherein the priming dose comprises

a composition comprising anti-CD4 monoclonal antibody and an immunomodulator, and wherein the priming dose modulates the individual's immune system to respond with induction of a cell mediated immune response comprising a cytotoxic **CD8+ T cell** response.

64. A vaccination kit comprising in separate containers: (a) a priming dose comprising a composition selected from the group consisting of an immunotherapeutic composition, anti-CD4 monoclonal antibody, and a combination thereof; and (b) an immunizing dose comprising tumor-associated antigen.

65. The vaccination kit according to claim 64, wherein the priming dose is contained in a sold phase implant for delivery of the composition comprising the priming dose over a desired period of time.

66. The vaccination kit according to claim 64, further comprising a component selected from the group consisting of an immunomodulator, a pharmaceutically acceptable carrier, and a combination thereof.

67. The vaccination kit according to claim 64, further comprising instructional material.

68. The vaccination kit according to claim 66, further comprising instructional material.

L14 ANSWER 21 OF 27 USPATFULL on STN
2001:178632 Method of using human receptor protein 4-1BB.
Kwon, Byoung S., Carmel, IN, United States
Advanced Research and Technology, Indianapolis, IN, United States (U.S. corporation)
US 6303121 B1 20011016
APPLICATION: US 1998-7097 19980114 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed herein are the methods of using the H4-1BB protein, ligands to this protein, and various mAbs either directed against H4-1BB or other molecules that can be used therapeutically. The nature and importance of the H4-1BB molecule provides the ligands and related co-stimulatory molecules the ability to enhance or **suppress** T-cell activation and proliferation. By treating T-cells that have expressed receptor protein H4-1BB with one of the four anti-H4-1BB monoclonal antibodies disclosed herein activation or **inhibition** of the immune response is seen. Also disclosed herein is cDNA for the human receptor H4-1BB. The cDNA of the human receptor H4-1BB is about 65% homologous to the mouse cDNA 4-1BB and was isolated by using probes derived from murine cDNA 4-1BB. A fusion protein for detecting cell membrane ligands to human receptor protein H4-1BB was developed. It comprises the extracellular portion of the receptor protein H4-1BB and a detection protein, alkaline phosphatase, bound to the portion of the receptor protein H4-1BB. B-cells that have expressed a ligand to receptor protein H4-1BB can be treated with cells that have expressed receptor protein H4-1BB and B-cell proliferation may be induced. The use of H4-1BB to block H4-1BB ligand binding has practical application in the **suppression** of the immune system during organ transplantation or against autoimmune diseases including diabetes, rheumatoid arthritis, and lupus. Other applications of this technology include the development of therapeutic methods for the treatment of **HIV**-1 infected individuals, and the treatment of cancerous tumors.

CLM What is claimed is:

1. A method of enhancing T-cell activation, comprising: administering an effective amount of an agonistic monoclonal antibody which binds to human 4-1BB (H4-1BB) such that said antibody comes into contact with at least one T-cell, thereby enhancing activation of said T-cell.

2. A method of enhancing T cell proliferation, comprising: administering

an agonistic monoclonal antibody which binds to H4-1BB effective to enhance T cell proliferation.

3. A method to enhance H4-1BB expression on T cells, comprising: administering an amount of an agonistic monoclonal antibody which binds to H4-1BB effective to enhance H4-1BB expression on T cells.

4. A method to **inhibit** apoptosis, comprising: administering an amount of an agonistic monoclonal antibody which binds to H4-1BB effective to **inhibit** apoptosis.

5. A method of any one of claims 1, 2, 3 or 4, wherein said antibody is of the IgG isotype.

6. The method of claim 1, 2, 3, 4 or 5 wherein said antibody is administered at a dosage range equivalent to or greater than 0.20 μmol to 2.0 μmol, one to three times per day.

7. The method of claim 6 wherein the administration of said antibody is accomplished through administration of a pharmaceutical formulation which is a tablet or intravenous injection.

8. The method of claim 1, 2, 3, 4 or 5 further comprising administering a second stimulatory molecule, in conjunction with said antibody such that both of these compounds come into contact with the same said at least one T-cell.

9. The method of claim 8, wherein said second stimulatory molecule is selected from the group consisting of: a) an anti-CD3 antibody; and b) an anti-CD28 antibody.

10. The method of claim 8, wherein said antibody is administered at a dosage range equivalent to or greater than 0.20 μmol to 2.0 μmol, one to three times per day, and wherein said second stimulatory molecule is administered at a dosage range equivalent to or greater than 0.10 μmol to 2.0 μmol, one to three times per day.

11. The method of claim 10, further comprising administering a third stimulatory molecule.

12. The method of claim 11, wherein the administration of said antibody, said second stimulatory molecule, and said third stimulatory molecule is accomplished through an administration of a pharmaceutical formulation which is a tablet or an intravenous injection.

13. The method of claim 11 wherein the second stimulatory molecule is an anti-CD3 antibody and the third stimulatory molecule is an anti-CD28 antibody.

14. The method of claim 8, wherein the administration of said antibody and said second stimulatory molecule is accomplished through an administration of a pharmaceutical formulation which is a tablet or an intravenous injection.

L14 ANSWER 22 OF 27 USPATFULL on STN
2001:116560 Methods of **inhibiting** CD40L binding to CD40 with soluble monomeric CD40L.
Armitage, Richard J., Bainbridge Island, WA, United States
Fanslow, William C., Federal Way, WA, United States
Spriggs, Melanie K., Seattle, WA, United States
Srinivasan, Subhashini, Kirkland, WA, United States
Gibson, Marylou G., Carlsbad, CA, United States
Immunex Corporation, Seattle, WA, United States (U.S. corporation)
US 6264951 B1 20010724
APPLICATION: US 1996-769819 19961219 (8)

DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB There is disclosed a polypeptide (CD40-L) and DNA sequences, vectors and transformed host cells useful in providing CD40-L polypeptides. More particularly, this invention provides isolated human and murine CD40-L polypeptides that bind to the extracellular binding region of a CD40 receptor. Also disclosed are methods of **inhibiting** undesirable immune responses, preventing T cell interaction with B cells by blocking CD40L binding to CD40 sites on B cells and other target cells.
CLM What is claimed is:
1. A method of **inhibiting** lupus, the method comprising administering a soluble monomeric CD40L polypeptide.

2. A method of preventing CD40L binding to CD40 sites on B cells and other target cells, the method comprising administering a CD40 antagonist, such that CD40L binding to CD40 sites on B cells and other target cells is prevented, wherein the CD40 antagonist is a soluble monomeric CD40-L polypeptide.

L14 ANSWER 23 OF 27 USPATFULL on STN
2000:102475 Universal donor cells.
Sims, Peter J., Mequon, WI, United States
Bothwell, Alfred L. M., Guilford, CT, United States
Elliot, Eileen A., New Haven, CT, United States
Flavell, Richard A., Killingworth, CT, United States
Madri, Joseph, North Branford, CT, United States
Rollins, Scott, Monroe, CT, United States
Bell, Leonard, Woodbridge, CT, United States
Squinto, Stephen, Irvington, NY, United States
Oklahoma Medical Research Foundation, Oklahoma City, OK, United States (U.S. corporation)Yale University, New Haven, CT, United States (U.S. corporation)
US 6100443 20000808
APPLICATION: US 1995-483433 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Genetically engineered cells are provided which can serve as universal donor cells in such applications as reconstruction of vascular linings or the administration of therapeutic agents. The cells include a coding region which provides protection against complement-based lysis, i.e., hyperacute rejection. In addition, the cell's natural genome is changed so that functional proteins encoded by either the class II or both the class I and the class II major histocompatibility complex genes do not appear on the cell's surface. In this way, attack by T-cells is avoided. Optionally, the cells can include a self-destruction mechanism so that they can be removed from the host when no longer needed.
CLM What is claimed is:
1. An isolated non-human mammalian cell expressing a nucleotide molecule which codes for human CD59 and expressing a nucleotide molecule which codes for a protein **inhibiting** complement mediated attack of said cell, the protein selected from the group consisting of human CD55 and CD46.

2. The cell of claim 1 wherein the cell expresses CD55.

3. The cell of claim 1 wherein the cell expresses CD46.

4. The cell of claim 1 wherein said cell is selected from the group consisting of endothelial cells, fibroblasts, epithelial cells, skeletal, cardiac and smooth muscle cells, hepatocytes, pancreatic islet cells, bone marrow cells, astrocytes, and Schwann cells.

5. The cell of claim 1 further comprising a nucleotide molecule which is expressed by the cell and which codes for bacterial cytosine deaminase.

6. The cell of claim 1 wherein the cell is selected from the group consisting of cells of bovine origin and cells of porcine origin.

7. A prosthesis for implantation having cells according to claim 1 attached thereto, said cells being resistant to complement mediated attack.

8. The prosthesis of claim 7 wherein the prosthesis is a vascular graft.

9. The prosthesis of claim 7 wherein the prosthesis is a stent.

10. A method for re-endothelializing a blood carrying vessel selected from the group consisting of denuded blood vessels, stents, and vascular grafts comprising applying to the vessel cells according to claim 1, said cells being resistant to complement mediated attack, thereby allowing re-endothelialization of said vessel.

11. A transgenic non-human mammal comprising cells expressing a nucleotide molecule which codes for human CD59 and expressing a nucleotide molecule which codes for a protein **inhibiting** complement mediated attack of said cells, the protein selected from the group consisting of human CD55 and CD46, wherein the CD59 and, CD55 or CD46 are expressed in an amount effective to **inhibit** complement mediated attack of the cells when introduced into a human.

12. An organ from a transgenic non-human mammal, the organs formed of cells expressing a nucleotide molecule which codes for human CD59 and expressing a nucleotide molecule which codes for a protein **inhibiting** complement mediated attack of said cells, the protein selected from the group consisting of human CD55 and CD46, wherein the CD59 and, CD55 or CD46 are expressed in an amount effective to **inhibit** complement mediated attack of the cells when introduced into a human.

13. An isolated non-human mammalian tissue comprising non-human mammalian cells, the cells expressing a nucleotide molecule which codes for human CD59 and expressing a nucleotide molecule which codes for a protein **inhibiting** complement mediated attack of said cells, the protein selected from the group consisting of human CD55 and CD46, wherein the CD59 and, CD55 or CD46 are expressed in an amount effective to **inhibit** complement mediated attack of the cells when introduced into a human.

14. The tissue of claim 13 wherein the cells express CD55.

15. The tissue of claim 13 wherein the cells express CD46.

16. The tissue of claim 13 comprising cells selected from the group consisting of endothelial cells, fibroblasts, epithelial cells, skeletal, cardiac and smooth muscle cells, hepatocytes, pancreatic islet cells, bone marrow cells, astrocytes, and Schwann cells.

17. The tissue of claim 13 comprising cells selected from the group consisting of cells of bovine, and porcine origin.

L14 ANSWER 24 OF 27 USPATFULL on STN
2000:57345 Surrogate tolerogenesis for the development of tolerance to xenografts.
Beschorner, William E., Baldwin, MD, United States
Ximerex, Inc., Omaha, NE, United States (U.S. corporation)
US 6060049 20000509
WO 9427622 19931208
APPLICATION: US 1995-295899 19950606 (8)
WO 1994-US5844 19940524 19950606 PCT 371 date 19950606 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a method for developing immune tolerance in xenogeneic organ graft recipients, in which lympho-hematopoietic cells from an intended organ graft recipient are differentiated within a xenogeneic surrogate, such as a fetal animal. After birth of the surrogate, the matured lympho-hematopoietic cells containing antigen specific regulatory cells, including **suppressor** cells, veto cells, select B cells, anti-idiotype antibodies, and other related factors responsible for antigen specific tolerance in a surrogate animal are reintroduced into the intended organ graft recipient, in conjunction with an organ transplant or a tissue transplant from the xenograft surrogate. The invention also provides an organ graft repopulated with cells from the intended organ graft recipient produced in a surrogate animal.

CLM What is claimed is:

1. A method for xenograft transplant of an organ to a recipient mammal from a donor mammal, wherein said recipient has increased immuno tolerance to donor tissue, comprising the steps of: a) collecting a first cell population from the recipient, said first cell population containing lymphocytic progenitor cells, wherein at least a portion of cells that are specifically cytotoxic to tissue from a surrogate animal are removed from said first cell population; b) administering said first cell population to said surrogate, said surrogate being in a state of immune deficiency; c) developing in said surrogate a state of immune competence; d) collecting from said immune competent surrogate a second population of cells, said second cell population containing immunocompetent cells, said immunocompetent cells specifically **suppressing** immune response of said recipient to tissue of said surrogate; e) infusing said second population of cells into said recipient; f) excising an organ from a donor mammal wherein the donor mammal is selected from the group consisting of the same surrogate mammal, the same inbred strain as the surrogate mammal and a littermate of the surrogate mammal; and g) transplanting said organ into said xenograft recipient.

2. The method of claim 1, wherein said first cell population from the recipient comprises bone marrow cells.

3. The method of claim 1, wherein said surrogate is a fetal mammal or a neonatal mammal.

4. The method of claim 1 further comprising treating said recipient with an immunosuppressive agent before transplanting said organ into said xenograft recipient.

5. The method of claim 1, wherein said second population of cells comprises spleen cells.

6. The method of claim 1, wherein the organ from the donor animal comprises vascular tissues.

7. The method of claim 1 wherein the said donor mammal is the same inbred strain as the surrogate mammal.

8. The method of claim 1 wherein the said donor mammal is a littermate of the surrogate mammal.

9. A method of transplanting an organ from a donor mammal to a recipient mammal which is not syngeneic with said donor mammal, comprising a) administering to said recipient mammal a cell population containing immunocompetent cells specifically **suppressing** immune response of said recipient mammal to said donor mammal, wherein said cell population is obtained from a surrogate mammal, said surrogate mammal being a chimeric animal containing lymphocytes derived from said recipient mammal; and b) transplanting an organ from said donor mammal to said recipient mammal, wherein said donor mammal is selected from the group consisting of the same surrogate mammal, the same inbred strain as the surrogate mammal,

and a littermate of the surrogate mammal, whereby immune response of said recipient mammal to said organ is reduced as compared to the immune response of said recipient mammal in the absence of the administration of said surrogate mammal cell population.

10. The method of claim 9, wherein said surrogate mammal and said recipient mammal are from different species.

11. The method of claim 10, wherein said surrogate mammal and said donor mammal are different individual mammals.

12. The method of claim 10, wherein said donor mammal and said recipient mammal are from the same species.

13. The method of claim 9, wherein said donor mammal is mature when said surrogate mammal is born.

14. The method of claim 9, wherein said donor mammal and said surrogate mammal are different individuals and said organ is a population of cells, further wherein said cell population is expanded by culturing in said surrogate mammal before transplanting into said recipient mammal.

15. The method of claim 14, wherein said organ is selected from the group consisting of pancreatic islet cells and hepatic cells.

16. The method of claim 14, wherein said organ is bone marrow.

17. A method of transplanting an organ from a third party donor mammal to a recipient mammal which is of the same species as said third party donor mammal, comprising a) administering to said recipient mammal a cell population containing immunocompetent cells specifically **suppressing** immune response of said recipient mammal to said third party donor mammal, wherein said cell population is obtained from a surrogate mammal, said surrogate mammal being a chimeric mammal containing lymphocytes derived from a set of mammals of the same species as the recipient, said set of mammals having a plurality of tissue types; and b) transplanting an organ from said third party donor mammal to said recipient mammal, whereby immune response of said recipient mammal to said organ is reduced as compared to the immune response of said recipient mammal in the absence of the administration of said surrogate mammal cell population.

18. A kit for **suppression** of immune rejection by a recipient mammal of an organ transplanted from a third party donor mammal which is of the same species as the surrogate mammal comprising an immune **suppressive** composition comprising a cell population obtained from a surrogate mammal, said surrogate mammal being a chimeric mammal containing lymphocytes derived from said recipient mammal and expressing antigens substantially identical to said third party donor mammal, and said cell population containing immunocompetent cells specifically **suppressing** immune response of said recipient mammal to said third party donor mammal, said cell population being suspended in a medium suitable for injection into said recipient mammal.

19. The kit of claim 18, wherein said immune **suppressive** composition is substantially free of immune reactive cells of said surrogate.

20. The kit of claim 18, wherein the composition suitable for injection into said recipient mammal is free of infectious agents.

21. A kit for organ transplant into a recipient mammal comprising an excised bodily organ from a surrogate mammal wherein said surrogate is different from said recipient and perfusion solution in an amount sufficient to preserve said excised organ in condition suitable for transplant into said recipient mammal, wherein a plurality of resident cells of said organ are cells from the same species as said recipient,

said resident cells being selected from endothelial cells, monocytes, dendritic cells, and lymphoid cells, said bodily organ being placed in the perfusion solution and preserved in condition suitable for transplant into said recipient mammal.

22. The kit of claim 21, wherein said plurality of resident cells are syngeneic with said recipient mammal.

23. A method of preparing an excised organ of claim 21 for transplant into a recipient mammal which is not syngeneic with said excised organ, comprising: a) collecting a cell population from said recipient mammal, said first cell population containing lymphocytic progenitor cells; b) administering said cell population to a surrogate mammal, said surrogate being in a state of immune deficiency; c) developing in said surrogate a state of immune competence; d) excising from said immune competent surrogate an organ, said organ being populated with at least a plurality of cells derived from said recipient mammal; and e) placing said organ in perfusion solution so that said excised organ is preserved in condition suitable for transplant into said recipient mammal.

24. The method of claim 23, further wherein at least a portion of cells that are specifically cytotoxic to tissue from said surrogate mammal are removed from said cell population before said cell population is administered to said surrogate.

25. A method of preparing an excised organ for transplant into a recipient mammal which is not syngeneic with said excised organ, comprising: a) collecting a cell population from an animal which is of the same species as said recipient mammal, said cell population containing lymphocytic progenitor cells; b) administering said cell population to a surrogate mammal, said surrogate being in a state of immune deficiency; c) developing in said surrogate a state of immune competence; d) excising from said immune competent surrogate an organ, said organ being populated with a plurality of cells derived from said recipient mammal; and e) placing said organ in perfusion solution so that said excised organ is preserved in condition suitable for transplant into said recipient mammal.

26. The method of claim 25, further wherein at least a portion of cells that are specifically cytotoxic to tissue from said surrogate mammal are removed from said cell population before said cell population is administered to said surrogate.

27. The method of claim 25, wherein lymphocytes derived from a set of mammals of the same species as the recipient are administered to said surrogate mammal while it is in a state of immune deficiency, said set of mammals having a plurality of tissue types.

28. The method according to claim 27, wherein said recipient mammal is a human having severe burns, said surrogate mammal is a non-human mammal, and said organ is a graft of skin from said non-human mammal for application to the burned area of said recipient, wherein a plurality of resident cells of said skin are cells derived from the same species as said patient.

29. The method of claim 25, wherein said donor mammal and said surrogate mammal are different individuals and said organ is a population of cells, further wherein said cell population is expanded by culturing in said surrogate mammal before transplanting into said recipient mammal.

30. The method of claim 29, wherein said organ is selected from the group consisting of pancreatic islet cells and hepatic cells.

31. The method of claim 29, wherein said organ is bone marrow.

32. A method of therapy for a patient having fulminant hepatitis,

comprising periodically transplanting hepatic cells prepared according to claim 30 into said patient so long as hepatic necrosis continues.

33. A method of **suppressing** specific immune response of a recipient human to a transplantable organ of a non-human mammal comprising administering to the recipient human, prior to transplantation of the organ of a non-human mammal, a cell population containing immunocompetent cells specifically **suppressing** immune response of the recipient human to the organ of the non-human mammal, the cell population being obtained from a non-human surrogate mammal, the non-human surrogate mammal being a chimeric animal containing said immunocompetent cells specifically **suppressing** human immune response to cells from the non-human mammal, the said immunocompetent cells being derived from the recipient human, whereby immune response of the recipient human to the organ is reduced as compared to the immune response of said recipient mammal in the absence of the administration of said surrogate mammal cell population.

34. The method of claim 33, further wherein at least a portion of cells that are specifically cytotoxic to tissue from said surrogate mammal are removed before cells are administered to said surrogate.

35. The method of claim 33, further comprising, before the step of collecting said cell population, a step of collecting fresh lymphocytes from said recipient and infusing said fresh lymphocytes into said developed surrogate.

36. The method of claim 33, wherein said surrogate is a fetal mammal.

37. The method of claim 33, wherein the surrogate is selected from the group consisting of a newborn mammal, a juvenile mammal and an adult mammal, said mammal having received lethal irradiation or chemotherapy followed by bone marrow transplant of a cell population comprising hematopoietic and lymphoid cells from an organism selected from said recipient mammal, said donor mammal, and said surrogate, or a mixture of hematopoietic and lymphoid cells from two or more of these organisms.

38. The method of claim 33, wherein said surrogate is the same mammal as said donor mammal.

39. The method of claim 33, wherein said lymphocytes are additionally administered to a plurality of immune deficient surrogates and said plurality of surrogates is allowed to develop into immune competent organisms.

40. The method of claim 39, further comprising, before the step of collecting said cell population of claim 17, the steps of: i) collecting respective blood or spleen samples from the plurality of immune competent developed surrogates; ii) testing the respective blood samples to determine a degree of engraftment with cells of said recipient mammal and to determine a degree of maturation of lymphocytes in the respective blood samples; iii) testing respective cells of the plurality of developed surrogates for specific **suppression** of immune reactivity between recipient mammal cells and donor mammal cells; iv) selecting, in response to the steps of testing the respective blood samples, a selected set of the plurality of developed surrogates, said selected set having the greatest degree of engraftment and the greatest immune **suppression**; v) testing for graft-vs-host disease (GvHD) against respective developed surrogates of the selected set by collecting fresh lymphocytes from said xenograft recipient and infusing said fresh lymphocytes into the selected set of developed surrogates; and vi) identifying a best developed surrogate from the selected set of developed surrogates, based on least degree of GvBD in response to infusion of lymphocytes from the xenograft recipient.

41. The method of claim 33, wherein lymphocytes derived from both

genetic parents of said donor mammal are administered to said surrogate mammal while it is in a state of immune deficiency.

42. The method of claim 41, wherein said organ is a cell suspension, and further wherein the cell suspension contains cells from more than one off-spring of said genetic parents.

43. The method of claim 41, wherein said donor mammal is mature when said surrogate mammal is born.

44. The method of claim 33, wherein said donor mammal and said surrogate mammal are different individuals and said organ is a cell suspension, further wherein said cell suspension is expanded by culturing in said surrogate mammal before transplanting the expanded cell suspension into said recipient mammal.

45. The method of claim 44, wherein said organ is selected from the group consisting of pancreatic islet cells and hepatic cells.

46. The method of claim 44, wherein said organ is bone marrow.

47. The method of claim 33, wherein the immunocompetent cells are human lymphocytes derived from hematopoietic cells of the recipient human introduced into the surrogate animal when the surrogate mammal was in a state of immune deficiency, the immunocompetent cells being collected from the surrogate mammal after the surrogate mammal achieved a state of immune competency.

48. The method of claim 33, wherein the non-human surrogate mammal is a primate, an artiodactyl or a carnivore.

49. The method of claim 33, wherein the non-human surrogate mammal is a primate or a pig.

50. The method of claim 33, wherein the non-human surrogate mammal is a rodent or a lagomorph, and the transplantable organ is a suspension of cells.

51. A method of preparing an excised non-human organ suitable for transplant into a recipient human comprising: a) collecting a human cell population containing lymphocytic progenitor cells; b) administering the cell population to a non-human donor mammal, the mammal being in a state of immune deficiency; c) developing in the non-human mammal a state of immune competence; d) excising an organ from the immune competent non-human mammal, the organ being populated with a plurality of cells derived from the human cell population; and e) placing the organ in perfusion solution so that the excised organ is preserved in condition suitable for transplant into a recipient human.

52. The method of claim 51, wherein the non-human mammal is a primate, an artiodactyl or a carnivore.

53. The method of claim 51, wherein the non-human surrogate mammal is a primate or a pig.

54. The method of claim 53, wherein the non-human mammal in a state of immune deficiency is a fetal mammal.

55. The method of claim 51, wherein the human cell population is obtained from the recipient human.

L14 ANSWER 25 OF 27 USPATFULL on STN
1998:68869 T cell differentiation of CD34+ stem cells in cultured thymic epithelial fragments.
Ruiz, Margaret Eileen, 4202 E. West Highway, Chevy Chase, MD, United States

20815
US 5766944 19980616
APPLICATION: US 1996-775509 19961231 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A procedure for carrying out T cell differentiation of CD34+ stem cells in an in vitro culture of thymic epithelial fragments whereby the differentiated T cells achieve full immunocompetence. The invention also includes the procedure for differentiation of stem cells from **HIV** seropositive individuals or genetically modified stem cells. The invention broadly relates to the culture of cultured thymic epithelial fragments and provides procedures for verifying true immunocompetence of the resulting T cells and for analyzing the effects of various compounds on the differentiation process. The invention also comprises several novel applications for utilizing the procedure of the invention, including grafting fortified cultured thymic epithelial fragments and infusing immunocompetent T cells into patients with compromised immune systems.

CLM What is claimed is:

1. A process of coculturing Cultured Thymic Epithelial Fragments and bone-marrow derived stem cells whereby the stem cells are differentiated into immunocompetent T cells, comprising the following steps: establishing cultured thymic epithelial fragment cultures by: excising thymus tissue as a metabolic specimen avoiding mechanical pressure and heat cauterization and placing the tissue in chilled media, removing the thymic capsule from the excised thymic tissue, mincing the tissue into small fragments and agitating the fragments in complete media to wash out thymocytes, depleting thymocytes and hematopoietic cells including dendritic cells by incubating the fragments on sterile tissue rafts which are partially immersed in complete medium supplemented with 2'-deoxyguanosine, at about 37° C. in a partial CO_2 atmosphere, and culturing the T cell depleted fragments to optimize stromal viability in complete medium supplemented with hydrocortisone, epidermal growth factor, cholera enterotoxin, insulin, adenine, and sodium pyruvate, isolating CD34+CD38- stem cells by: performing Ficoll-Hypaque density centrifugation to obtain nucleated bone marrow cells from bone marrow aspirates diluted in normal saline, depleting CD2+ lymphocytes by rosette formation with sheep Red Blood Cells treated with 2-amino-ethyl isothiouronium bromide, followed by a second Ficoll-Hypaque density centrifugation, removing non-CD34 cells by culture with monoclonal antibodies or immunomagnetic microbeads, followed by separation over a monoclonal antibody-coated panning flask or magnetic column, and coculturing the Cultured Thymic Epithelial Fragments and purified CD34+CD38- Stem Cells by: seeding CD34+CD38- stem cells by infusion onto the surface of the Cultured Thymic Epithelial Fragments in transwell plates in Iscove's/Ham's medium containing IL-2 at about 37° C. in a partial CO_2 atmosphere until differentiated into mature T cells, replacing medium every few days, and isolating the differentiated T cells by aspirating the cells from the culture wells, washing, resuspending in saline, subjecting the cells to Ficoll-Hypaque density centrifugation to remove dead cells, washing the mononuclear cells, and resuspending the pure mononuclear cells in saline.

2. A process of coculturing Allogeneic Cultured Thymic Epithelial Fragments and bone-marrow derived stem cells from **HIV** seropositive patients whereby the stem cells are differentiated into immunocompetent T cells free of **HIV** infection, comprising the following steps: establishing cultured thymic epithelial fragment cultures by: excising thymus tissue as a metabolic specimen avoiding mechanical pressure and heat cauterization and placing the tissue in chilled media, removing the thymic capsule from the excised thymic tissue, mincing the tissue into small fragments and agitating the fragments in complete media to wash out thymocytes, depleting thymocytes and hematopoietic cells including dendritic cells by incubating the fragments on sterile tissue rafts which are partially immersed in complete medium supplemented with

2'-deoxyguanosine, at about 37° C. in a partial CO_2 atmosphere for at least seven days, and culturing the T cell depleted fragments to optimize stromal viability in complete medium supplemented with hydrocortisone, epidermal growth factor, cholera enterotoxin, insulin, adenine, and sodium pyruvate, isolating CD34+ stem cells from **HIV** seropositive patients by: collecting bone marrow from aspirates in Heparinized tubes, performing Ficoll-Hypaque density centrifugation to obtain nucleated bone marrow cells from bone marrow aspirates diluted in normal saline, depleting CD2+ lymphocytes by rosette formation with sheep Red Blood Cells treated with 2-amino-ethyl isothiouronium bromide, followed by a second Ficoll-Hypaque density centrifugation, removing macrophage/monocytic cells by adherence on plastic Petri dishes, removing non-CD34 cells by culture with monoclonal antibodies or immunomagnetic microbeads, followed by separation over a monoclonal antibody-coated panning flask or magnetic column, and coculturing the Cultured Thymic Epithelial Fragments and purified CD34+CD38- Stem Cells by: seeding a minimum of 5,000 lineage negative stem cells or 50,000 lineage committed stem cells by infusion onto the surface of the Cultured Thymic Epithelial Fragments in transwell plates in Iscove's/Ham's medium containing IL-2 at about 37° C. in a partial CO_2 atmosphere until differentiated into mature T cells, replacing medium every few days, and isolating the differentiated T cells by aspirating the cells from the culture wells, washing, resuspending in saline, subjecting the cells to Ficoll-Hypaque density centrifugation to remove dead cells, washing the mononuclear cells, and resuspending the pure mononuclear cells in saline.

3. A process whereby thymic microenvironmental performance (i.e. thymopoiesis) may be evaluated comprising the step of carrying out the process of claim 1 or claim 2 in the presence of a compound proposed to be used for **HIV** therapy to determine if the stem cell differentiation is **inhibited** or otherwise altered in the presence of said compound.

4. A process of expanding and differentiating bone marrow-derived stem cells into immunocompetent T cells comprising the step of coculturing the stem cells in an in vitro Cultured Thymic Epithelial Fragment 3-D microenvironment which comprises more than an Thymic Epithelial Cell Monolayer.

L14 ANSWER 26 OF 27 USPATFULL on STN
1998:2010 Universal donor cells.
Sims, Peter J., Mequon, WI, United States
Bothwell, Alfred L.M., Guilford, CT, United States
Elliot, Eileen A., New Haven, CT, United States
Flavell, Richard A., Killingworth, CT, United States
Madri, Joseph, North Branford, CT, United States
Rollins, Scott, Monroe, CT, United States
Bell, Leonard, Woodbridge, CT, United States
Squinto, Stephen, Irvington, NY, United States
Oklahoma Medical Research Foundation, Oklahoma City, OK, United States (U.S. corporation)Yale University, New Haven, CT, United States (U.S. corporation)
US 5705732 19980106
APPLICATION: US 1993-87007 19930701 (8)
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Genetically engineered cells are provided which can serve as universal donor cells in such applications as reconstruction of vascular linings or the administration of therapeutic agents. The cells include a coding region which provides protection against complement-based lysis, i.e., hyperacute rejection. In addition, the cell's natural genome is changed so that functional proteins encoded by either the class II or both the class I and the class II major histocompatibility complex genes do not appear on the cell's surface. In this way, attack by T-cells is avoided. Optionally, the cells can include a self-destruction mechanism so that

they can be removed from the host when no longer needed.

CLM What is claimed is:

1. A transgenic non-human mammal all of whose nucleated cells contain a nucleotide sequence encoding human CD59, wherein CD59 is expressed on the surface of the mammal's cells capable of expressing human CD59, in an amount effective to **inhibit** complement mediated damage when the cells are exposed to human blood or serum and where the cells express at least 1×10^3 CD59 molecules per cell.

2. The transgenic mammal of claim 1 wherein the mammal also expresses a protein **inhibiting** complement selected from the group consisting of CD55 and CD46.

3. The transgenic mammal of claim 1 wherein the mammal is a pig.

4. The transgenic mammal of claim 1 wherein the CD59 molecule comprises the amino acid residues one to seventy seven set forth in SEQ ID NO. 3.

5. The transgenic mammal of claim 1 wherein the CD59 molecule is encoded by the nucleotide sequence set forth in SEQ ID NO. 4.

L14 ANSWER 27 OF 27 USPATFULL on STN

95:105692 Method for determining favorable prognosis in an **HIV** positive subject using HLA-DR+ /CD38$^-$ **CD8**^{bright} cells.

Giorgi, Janis V., Woodland Hills, CA, United States

Hultin, Lance, West Hills, CA, United States

The Regents of the University of California, Los Angeles, CA, United States (U.S. corporation)

US 5470701 19951128

APPLICATION: US 1993-22971 19930224 (8)

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AB The subject invention involves an approach to determining the favorable prognosis of a subject infected with **HIV**. Specifically, the discovery involves the importance of the elevated presence of MHC class II antigen+ /CD38$^-$ /**CD8**^{bright} cells for the prognosis of favorable outcome in **HIV**-infected subjects. In one example the MHC class II antigen may be HLA-DR.

CLM What is claimed is:

1. A method for determining a favorable prognosis in an **HIV** positive subject which comprises quantitatively detecting an elevated level of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells thereby determining a favorable prognosis in the **HIV** positive subject.

2. A method as claimed in claim 1, wherein in the elevated level of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells, the cells have relatively greater levels of HLA-DR antigen and relatively less CD38 antigen relative to a threshold for each of the HLA-DR and CD38 antigens, and applying a relationship between the elevated HLA-DR+ /CD38$^-$ /**CD8**^{bright} cell levels relative to the activated **CD8**^{bright} cells, the relationship being a diagnostic evaluation of the relatively favorable prognosis in the **HIV** positive subject.

3. A method as claimed in claim 1, wherein the elevated level of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells is determined relative to a number of activated **CD8**^{bright} cells present.

4. A method as claimed in claim 3, wherein a determination of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells constitutes a numerator in a relationship with the number of activated **CD8**^{bright} cells, such number of activated **CD8**^{bright} cells constituting a denominator in the relationship.

5. A method as claimed in claim 3, wherein a score of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells is determined and is represented as

a first cell count, and wherein a score of activated **CD8**^{bright} cells is determined and is represented as a second cell count such that when the number of cells in the first cell count relative to the number of cells in the second cell count is greater than a first discriminate value, this is determinative of a favorable prognosis.

6. A method as claimed in claim 3 including relating a percentage of the HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells relative to activated **CD8**^{bright} cells and applying a relationship of this percentage as a diagnostic evaluation of the condition.

7. A method as claimed in claim 3 wherein a favorable prognosis of the condition is determined in relation to an increased level of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells relative to the level of activated **CD8**^{bright} cells.

8. A method of diagnosing a stable disease condition associated with **HIV** in a human comprising: (a) obtaining a blood sample from the human; (b) testing the sample by determining at least one measure of an activated **CD8**^{bright} cell population having HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells; (c) applying a relationship between the HLA-DR+ /CD38$^-$ /**CD8**^{bright} cell population in relation to an activated **CD8**^{bright} cell population as a diagnostic evaluation of a favorable prognosis of the disease condition; and (d) presenting such relationship thereby diagnosing a stable disease condition associated with **HIV** in the human.

9. A method as claimed in claim 8 including determining a cell population with relatively greater HLA-DR antigen and relatively less CD38 antigen relative to a threshold for each of the HLA-DR and CD38 antigens, and applying this determination relative to the activated **CD8**^{bright} cells as a diagnostic evaluation of the stable disease condition associated with **HIV** infection in the human.

10. A method as claimed in claim 9, wherein a score of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells is determined and is represented as a first cell count, and wherein a score of activated **CD8**^{bright} cells is determined and is represented as a second cell count such that when the number of cells in the first cell count is greater than a first predetermined discriminate number and the number of cells in the second cell count is lower than a second predetermined discriminate number, this is determinative of a favorable prognosis.

11. A method as claimed in claim 9 wherein a score of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells is determined and is represented as a first cell count, such that when the number of cells in the first cell count is greater than a first predetermined discriminate number (1PDN), this is determinative of a favorable prognosis.

12. A method as claimed in claim 9 wherein a score of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells is determined and is represented as a first cell count, such that when the number of cells in the first cell count is greater than a second predetermined discriminate number (2PDN), this is determinative of a favorable prognosis.

13. A method as claimed in claim 8 including relating a percentage of the HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells relative to the percentage of activated **CD8**^{bright} cells, and applying a relationship of this percentage as a diagnostic evaluation of the stable disease condition associated with **HIV** in the human.

14. A method as claimed in claim 8, wherein a determination of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells constitutes a numerator in a relationship with the number of activated **CD8**^{bright} cells, such number of activated **CD8**^{bright} cells constituting a

denominator in the relationship.

15. A method as claimed in claim 8 including relating a percentage of the HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells relative to activated **CD8**^{bright} cells and applying a relationship of this percentage as a diagnostic evaluation of the stable disease condition associated with **HIV** disease in the human.

16. A method as claimed in claim 8 wherein a favorable prognosis of the condition is determined in relation to an increased level of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells relative to the level of activated **CD8**^{bright} cells.

17. A method for monitoring the course of disease in an **HIV** positive subject which comprises quantitatively determining in a first cell sample from the subject the presence of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells and comparing the amount so determined with the amount present in a second sample from the subject, such samples being taken at different points in time, a difference in the amounts determined being indicative of the course of disease.

18. A method as claimed in claim 17, wherein the presence of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells is determined in relation to a number of activated **CD8**^{bright} cells present.

19. A method of diagnosing a disease condition related to **HIV** in a human comprising: (a) obtaining a blood sample from the human, (b) determining from the sample at least one measure of an activated **CD8**^{bright} cell population having HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells, (c) presenting such measure, and (d) applying the measure of the HLA-DR+ /CD38$^-$ /**CD8**^{bright} cell population selectively as at least one of a diagnostic evaluation of a favorable prognosis of a disease condition, a stable disease condition or condition of **inhibition** of **HIV** replication.

20. A method as claimed in claim 19 including determining a cell population with relatively greater HLA-DR antigen and relatively less CD38 antigen relative to a threshold for each of the HLA-DR and CD38 antigens, and applying a measure of the HLA-DR+ /CD38$^-$ /**CD8**^{bright} cell population above the threshold relative to an activated **CD8**^{bright} cell population

21. A method as claimed in claim 20 including relating a percentage of the HLA-DR+ /CD38$^-$ /**CD8**^{bright} cell population of relatively greater concentration relative to a percentage of activated **CD8**^{bright} cells, and applying a relationship of this percentage as an evaluation of the condition.

22. A method as claimed in claim 19 including relating a percentage of the HLA-DR+ /CD38$^-$ /**CD8**^{bright} cell population of relatively greater concentration relative to a percentage of activated **CD8**^{bright} cells, and applying a relationship of this percentage as an evaluation of the condition.

23. A method as claimed in claim 14, wherein a favorable prognosis of the condition is determined in relation to an increased level of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells relative to activated **CD8**^{bright} cells.

24. A method as claimed in claim 19, wherein a determination of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells constitutes a numerator in a relationship with the number of activated **CD8**^{bright} cells, such number of activated **CD8**^{bright} cells constituting a denominator in the relationship.

25. A method of diagnosing a disease condition related to **HIV** in a human comprising: (a) obtaining a blood sample from the human, (b) determining from the sample at least one measure of an activated **CD8**^{bright} cell population having HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells in the sample, (c) applying a relationship between the HLA-DR+ /CD38+ /**CD8**^{bright} cell population in relation to a **CD8**+ lymphocyte population as a diagnostic evaluation of at least one of a favorable prognosis of the disease condition, a stable condition or **suppressor** of **HIV** replication; and (d) presenting such relationship.

26. A method as claimed in claim 25 including determining a cell population with relatively greater HLA-DR antigen and relatively less CD38 antigen relative to a threshold for each of the HLA-DR and CD38 antigens, and applying a measure of HLA-DR+ /CD38$^-$ /**CD8**^{bright} cells in a relationship with this threshold and relative to the activated **CD8**^{bright} cells as an evaluation of the disease condition.

27. A method of diagnosing a disease condition related to **HIV** infection in a human comprising: (a) obtaining a blood sample from the human, (b) determining from the sample at least one measure of a lymphocyte population having a HLA-DR+ /CD38$^-$ /**CD8**^{bright} cell, (c) applying a relationship between the HLA-DR+ /CD38$^-$ /**CD8**^{bright} cell population and the lymphocyte population as at least one of a diagnostic evaluation of the prognosis of a favorable disease condition, a stable condition or an **HIV suppression** condition, and (d) presenting such relationship.

28. The method as claimed in claim 25 or 27, wherein the measure is a ratio, percentage, absolute number, product, difference or quotient.

29. The method as claimed in claim 21, wherein the lymphocyte population is selected from a group consisting of a **CD8**+ cell, a **CD8**^{bright} cell, or an activated **CD8**^{bright} cell.